LIMNOLOGY AND OCEANOGRAPHY

July, 1957

VOLUME II

NUMBER 3

Chemical and Biological Studies on the Yellow Organic Acids of Lake Water[1]

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ABSTRACT

The yellow coloring matter of natural waters has been studied with particular emphasis on Lower Linsley Pond, Connecticut. The best method of isolation was by ethyl acetate extraction of acidified evaporation residues of filtered water.

The material thus obtained was a mixture of colorless and colored carboxylic acids, the colored ones fluorescing under ultra-violet light. Numerous yellow fractions were distinguished through paper chromatography, none of which showed maxima in either their visible or ultra-violet absorption spectra. Chemical tests indicated a phenolic or enolic character with probable unsaturation. Elemental analyses showed 1.4% ash, no halogens or sulfur, and resulted in an empirical formula of $(C_{50}H_{62}O_{27}N)_n$. The nitrogen was believed to be an impurity, resulting in an empirical formula of $C_{21}H_{26}O_{11}$ on the basis of the determined mean molecular weight of 456. The mean equivalent weight of the mixture was 228. The salts of the acids were dialysable and the acids were amorphous as shown by x-ray diffraction. Infra-red spectroscopy of various derivatives indicated a mixture of hydroxy carboxylic acids and admitted the possibility of unsaturation and aromaticity, although the indications are that if aromaticity exists it is relatively inconsequential. The materials were very resistant to chemical oxidation.

Lower Linsley Pond surface water contained between 4.5 and 9.0 milligrams of acids per liter, of which about 40% was estimated to be yellow. Raising the pH from 3 to 11 increased the color by 50%. Solutions of the salts obeyed Beer's law in the range studied from 5 to 70 mg/L. The materials may affect organisms through alteration of light and by their buffering action. They were reasonably stable to biological decomposition over a period of 4½ months. Concentrations of up to 50 mg/L had no effect on pure cultures of *Chlamydomonas Reinhardi*, but *Hematococcus pluvialis* and *Scenedesmus quadricauda* were stimulated in their growth by concentrations of between 5 and 50 mg/L. The materials were able to retain iron in a filterable state at pH values up to 13.2.

Eleven lakes showed very similar chromatographic patterns on the same date and on different dates. Evidence is presented for the hypothesis that some of the numerous colored fractions are different salts of a small number of acids. It is concluded that the materials originate as a result of decomposition in soil and possibly in sediments as well. The name 'humolimnic acids' is suggested for the materials to indicate their probable relation but improbable equivalence with so-called 'humic' acids.

INTRODUCTION

An understanding of the relationships existing between aquatic organisms and their environment must be based upon a consideration of all factors operating in that environment. One such factor, the dissolved organic matter, is almost wholly uninvestigated, and the purpose of this paper is to describe the results of a study of one fraction of this matter—the yellow substances responsible for the color of natural waters.

The nature and role of the yellow materi-

[1] Based on a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, Yale University, 1957. The present address of the author is Department of Zoology, University of Washington, Seattle 5, Washington.

als have been the subject of much speculation but the actual amount of pertinent information is very limited. Aschan (1908, 1932) working with material which he precipitated from Finnish lakes showed it to be acidic and to contain carbon, hydrogen, oxygen, a small amount of nitrogen and possibly phosphorus and sulfur. The observation by Birge and Juday (1934) that the carbon/nitrogen ratio is larger in more highly colored lakes tended to support the idea that the colored compounds are of low nitrogen content. The physical nature of the substances has been investigated by indirect means, and the general consensus has been that they are colloidal (James and Birge 1938, James 1941, Ohle 1934, 1940). In addition to this rather scant knowledge it is known that sunlight will bleach the color (Whipple 1927) and that the color is reversibly lightened by lowering the pH (Ohle 1934, Hutchinson, in press). Several investigators (Taylor 1949, Whipple 1927, Juday and Birge 1933) have noted increases following rain, and this suggests a soil origin.

The significance of the materials with regard to the penetration of light in lake waters was well demonstrated by Juday and Birge (1933), and Aschan (1908, 1932) discussed their role in the formation of lake iron ore and suggested a possible nutrient function. The relation of the compounds to the iron metabolism of lakes was indicated also by Åberg and Rodhe (1942), and Ohle (1933) suggested that the rich phytoplankton of certain humus lakes could be attributed to stimulation by these substances. More recently Braus (1951) and his coworkers have taken up the problem, and attention is being focussed on the colored materials in sea water through the researches of Kalle (1949), Jerlov (1953) and Johnston (1955).

The work presented here is a description of the isolation, analysis, and testing for various roles of the colored materials of lake water. Most of the work was done using surface water collected from Lower Linsley Pond, Connecticut, but enough has been done to show that the results are of more than local significance.

I am happy to acknowledge the assistance and support of the members of my PhD committee, Dr. Edward S. Deevey, Dr. Emil H. White, and particularly Professor G. Evelyn Hutchinson. Financial assistance was provided by the National Science Foundation and the Society of Sigma Xi. Opportunity to use the infra-red and ultraviolet spectrophotometers of the Sterling Chemistry Laboratory at Yale is particularly appreciated, as is the help of Dr. Kurt Servos and the use of the X-ray diffraction apparatus of the Yale Geology Department.

METHODS EMPLOYED

Samples of water, ranging in volume from 1 to 160 liters, were filtered within twenty-four hours after collection through a single layer of Whatman № 1 filter paper and then through a porcelain filter candle with a maximum pore diameter of 3 microns. Tests with Millipore filters having pore diameters of 0.45 micron showed almost no material left in suspension.

Recovery of the colored materials was possible by several techniques. Freezing-out resulted in satisfactory concentration but was not applicable to the large volumes required. (It does occur naturally however during winter.) Uptake by anion exchange resins in their hydroxide form was highly efficient, but subsequent recovery through elution was low. Cation exchange resins were totally ineffective. An alternative method was to acidify the water to pH 3 or below using hydrochloric or acetic acid and pass it through a bed of fairly coarse (60–90 mesh) Florisil—a magnesia-silica gel manufactured by the Floridin Company, Tallahassee, Florida. Although initial uptake was not quantitative nearly all of the adsorbed color could be eluted with 5% ammonium hydroxide or with 95% ethanol followed by the ammonia.

Most of the time however the procedure was as follows. The filtered water was evaporated under vacuum either in a small-batch-type glass still of conventional design, or in a glass and stainless steel continuous-feed-type rapid-circulating still patterned after that of van Heyningen (1949). This was modified from the original in being heated electrically, but the temperature of the water never rose above 45°C. Following evaporation the residues dried either

over steam or in an oven at 60°C were ground with 80% ethanol made acid with HCl. This procedure although leaving behind a moderate amount of colored material effectively removed much of the inorganic salt. Material "purified" to this extent is referred to below as 80% ethanol extract.

Further concentration and purification was accomplished by drying the 80% ethanol extract, redissolving it in dilute aqueous HCl, and extracting it in a separatory funnel with an organic solvent. Ethyl acetate extracted the most color, followed in efficiency by ethyl ether. Non-polar solvents such as benzene or pentane were of no value. No extraction took place from basic solution, and the smaller the volume of the acid solution the more complete the extraction. The colored materials were next shaken into a small volume of dilute potassium or sodium bicarbonate and washed with several changes of ethyl acetate to remove neutral substances. Following acidification the yellow materials were once more extracted into fresh ethyl acetate, the latter distilled off, and the residue either taken up in absolute methanol or dried in a vacuum oven at 40°C. The final product is referred to below as ethyl acetate extract or "free acids." Two such preparations contained 1.4% ash and 0.0% ash, respectively.

Paper chromatograms were run using conventional techniques. Most of the chromatograms were run by the descending method, and application of the materials to the paper as lines rather than as spots resulted in better resolution. The most useful paper was Whatman #3. All solvents were redistilled before use, and the most generally useful solvent mixture had the following composition by volume: pyridine 25, water 25, tertiary amyl alcohol 25, diethylamine 1. A second solvent used for rechromatogramming eluted materials was composed of methyl ethyl ketone 37.5, water 15, formic acid 7.5. The hydroxamic acid derivatives prepared for the detection of colorless acids were chromatogrammed using a mixture of equal volumes of secondary butyl alcohol and water. The yellow materials were found on the paper by their fluorescence under long wave ultra-violet light.

Advantage was taken of the fluorescence under u.v. light in making photographic copies of the chromatograms. Satisfactory results were obtained using Kodak Royal Pan film and a K2 (yellow) filter at F8 for about 60 seconds.

Absorption spectra in the visible and ultraviolet ranges were obtained with a Cary recording spectrophotometer, and the infra-red spectra were obtained with a Perkin-Elmer recording infra-red spectrophotometer. Because of the small quantities of materials involved all infra-red spectra were made with the potassium bromide technique.

Materials used to determine molecular and equivalent weights were weighed in the solid state with a microbalance.

RESULTS

Unless otherwise noted the work was carried out on samples from the surface waters of (Lower) Linsley Pond. As mentioned previously, "free acids" refers to the material obtained by extraction with ethyl acetate.

General properties

Physical appearance, melting point, solubility. The dried ethyl acetate extract forms a deep orange film which is clear by transmitted light and which is easily chipped and ground to a dark brown powder.

The free acid preparation begins to soften at about 95°C and becomes liquid by 150°C, decomposing at higher temperatures. The absence of a sharper melting point indicates the presence of several compounds.

The free acids are very slightly acid soluble, moderately water soluble, and very soluble in basic solution. They are insoluble in such non-polar solvents as benzene or pentane, moderately soluble in ether, ethyl acetate, or ethyl alcohol, and very soluble in methyl alcohol. The salts are extremely water soluble but insoluble in solvents other than aqueous alcohol. The methyl esters, prepared with diazomethane, have solubilities in organic solvents similar to those of the free acids, but are insoluble in acid, water, or sodium bicarbonate solution. The solubility relationships indicate a polar material.

Homogeneity. The exact number of yel-

TABLE 1. *Chromatographic zones of 80% ethanol extracts of lake water residues*

R_F	Color	Fluorescence	Remarks
00	yellow	yellow-green	
.11–.16	yellow front edge	yellow-green	can be split into two discrete zones
	orange back edge	yellow-orange	
.25	yellow	yellow-green	
.38–.56	yellow	yellow-green	broad and diffuse
.71–.80	yellow	yellow-green	may have 3 parts
.81	yellow	none	found twice
.94	yellow	yellow-green	found twice

low compounds present in the water is not known. The problem is complicated by the fact that at present the only criterion for distinguishing between different fractions is through their behavior on paper chromatograms.

Numerous chromatograms of the 80% ethanol extracts run with the pyridine solvent have given the same general results (Fig. 12). Usually about 5 or 6 yellow fluorescing zones were present. On occasion 1 yellow non-fluorescing zone was observed and this, coupled with the fact that of the other zones one may be double and another have 3 components, allows for about 10 chromatographically different yellow substances. Table 1 lists the zones and their properties.

Similarly, the colored materials when extracted from ethyl acetate with potassium bicarbonate solution and chromatogrammed on Whatman № 3 paper with the pyridine solvent separated into about 9 zones (Fig. 1). The properties of these zones are given in Table 2. On one occasion the zone with R_F 0.05 was eluted and rechromatogrammed using the methyl ethyl ketone solvent. The result is shown in Figure 2. About 6 zones are present. Assuming that all of the yellow fluorescing zones in Table 2 had at least 6 components, 42 different fractions might be present in the ethyl acetate extract.

It is clear then that a considerable number of yellow materials is present in the water. The reasons for the multiplicity are discussed in the section titled "true nature of the fractions."

Color. The material in solution has a color ranging from light yellow to black depending upon the concentration. The

FIG. 1. Chromatogram of a potassium bicarbonate extract of an ethyl acetate solution of the free acids. Direction of solvent flow is from top to bottom.

absorption spectrum between 3000 Å and 8000 Å is shown in Figure 3. The spectrum is the same in acid or alkaline solution or in ether.

Since this investigation was concerned primarily with the yellow materials, it was of interest and importance to determine whether the preparations consisted entirely of these materials or whether colorless sub-

TABLE 2. *Chromatographic zones of potassium bicarbonate extract of ethyl acetate extract of lake water residues*

R_F	Color	Fluorescence	Remarks
00	yellow	yellow-green	
.05	yellow	yellow-green	heaviest zone
.16	yellow	yellow-green	broad
.26	yellow	yellow-green	
.45	yellow	yellow-green	
.70	yellow	yellow-green	
.75	none	none	seems to absorb u.v.
.81	yellow	yellow-green	
.90	yellow	brownish	

stances were present also. To this end the acids were converted to their methyl esters and thence to their hydroxamic acid derivatives, as described by Thompson (1951). Such derivatives will react with ferric chloride under acid conditions to give deeply colored complexes. Thus ferric chloride treatment of a chromatogram of the hydroxamic acids would reveal the presence of colorless acids through the formation of complexes in areas other than those previously noted to be yellow. Figure 4 shows the result of such an experiment. The ferric chloride treatment has resulted in the formation of a colored complex in an area which was not yellow.

Thus far it has not been possible to devise a method that will tell what fraction of the material is colorless. Several other experiments similar to the one just described leave little doubt that a large fraction of the material is indeed colored. In any event many of the results to be discussed have been substantiated using material which was chromatogrammed twice. In other cases it is obviously the yellow acids whose properties were being observed even when colorless acids were known to be present.

Fluorescence. The yellow materials extracted from lake water, with one exception (Table 6), fluoresced under ultra-violet illumination, particularly under long wave ultra-violet. This property was used extensively throughout the investigation to follow the small quantities of material it was necessary to deal with. The fluorescence spectrum has not been determined, but the light emitted is greenish-yellow in

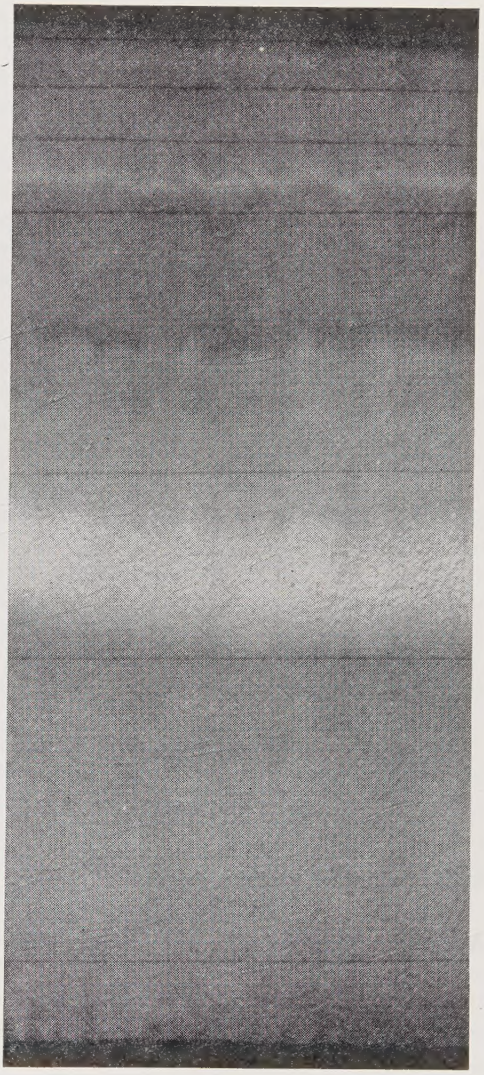

FIG. 2. Chromatogram of the material eluted from the zone with R_F 0.05 of the chromatogram in Figure 1. Direction of solvent flow is from top to bottom.

dilute solution and on paper. One chromatographic zone flouresces orange. Acid or alkali has no effect on the fluorescence, but prolonged oxidation with acid or alkaline permanganate will destroy it.

Acidity. The acid nature of the materials is evident from their behavior with respect to cation and anion exchange resins and their extraction from aqueous solution with organic solvents as well as from their solubility relationships with acid and base.

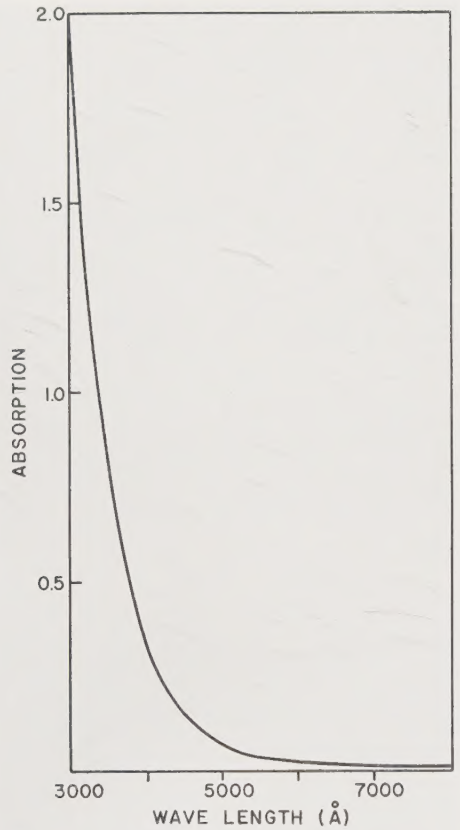

FIG. 3. Visible absorption spectrum of the colored materials isolated from lake water.

Additional evidence is that ethyl acetate extracts will turn blue litmus red. Extraction of ethyl acetate solutions with aqueous sodium bicarbonate which is commonly used to distinguish acids from phenols removed all of the color, showing that no weakly acidic colored phenols were present. Strongly acid phenols such as nitrophenols would go into the base along with the acids, but, as the infra-red analyses will show, the evidence is against the presence of such compounds. Further evidence for the presence of carboxyl groups is provided by the infra-red analyses and by conversion of the acids to their methyl esters through the use of diazomethane.

Tests for unsaturation. Treatment of twice chromatogrammed yellow acids directly with ferric chloride produced a color similar to the purple formed with hydroxamic acids but distinguishable from it. This suggested the presence of phenols or enols which are known to form colored iron complexes. Accordingly, the Ehrlich diazo reaction was applied (Feigl 1954), yielding a red dye distinctly different from the original color, which showed that enols or phenols were present.

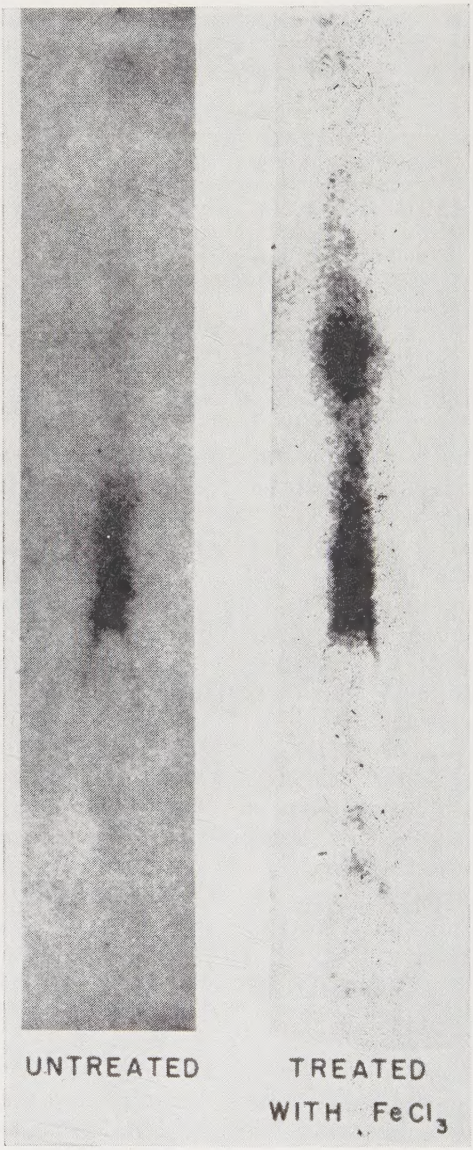

FIG. 4. Chromatogram of the hydroxamic acid derivatives before and after treatment with ferric chloride. The photograph at the left shows the yellow acids only. After treatment both yellow and colorless acids are visible. Direction of solvent flow is from bottom to top.

The presence of an enol or phenol would necessitate a degree of unsaturation. As will be shown later the data on infra-red and elemental analysis also allow for unsaturation, and the color of the materials indicates a system of conjugated double bonds. Further evidence of unsaturation comes from treatment with bromine water. If bromine water is added to an acidic solution of the yellow materials and the solution neutralized with sodium hydroxide after a few seconds, almost all of the color will have disappeared. (Traces of color still persist after two hours of treatment.) However, in spite of these indications direct microhydrogenation of the acids using platinum oxide catalyst resulted in no uptake of hydrogen even though it could be demonstrated that the catalyst had not been poisoned. Thus the problem is still unresolved.

Elemental analysis. A portion of the ethyl acetate extract was subjected to a Lassaigne sodium decomposition. Sulfur and the halogens were absent, but a small amount of nitrogen was found. Another portion of the same preparation was analyzed by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. On an ash-free basis (1.4% ash) the results were as follows: carbon 54.56%, hydrogen 5.64%, oxygen 39.12%, and nitrogen 1.23%. (Analysis of a second preparation revealed no ash, carbon 53.35%, hydrogen 5.38%.)

Assuming for the moment that the analysis represents a single nitrogen-containing compound, its empirical formula would be $(C_{50}H_{62}O_{27}N)_n$, and its minimum molecular weight would be 1115. However, since the molecular weight is much lower (see below), the nitrogen probably is present as an impurity. Recalculating the results on an ash-free and nitrogen-free basis results in an empirical formula of $(C_{1.86}H_{2.3}O)_n$, which on the basis of a molecular weight of 456 becomes $C_{21}H_{26}O_{11}$, and with an equivalent weight of 228 becomes $C_{19}H_{24}O_7(COOH)_2$. Assuming all of the remaining oxygen to be present in CHOH groups, the rest of the hypothetical molecule would be $C_{12}H_{10}$, showing a high degree of unsaturation. But if all the remaining oxygen is assumed to be in keto groups (C=O), then the remainder of the molecule would be $C_{12}H_{24}$ and there would be no unsaturation. Any real ideas as to the molecular structure must await further data.

Equivalent weight and molecular weight. It should be emphasized that since the preparations are mixtures and not pure compounds any values obtained for equivalent weight or molecular weight must be regarded as average values.

The determination of equivalent weight was made on 0.57 milligrams of dried free acids dissolved in 10 milliliters of distilled water. The initial pH was 3.9, and the solution was titrated with N/10 sodium hydroxide solution from a micro burette, and stirred with a nitrogen stream, the pH being determined with a pH meter after each addition of base. The resultant curve is shown in Figure 5. On the basis of the quantity of sodium hydroxide required to reach pH 7.0 the equivalent weight is 228.

The molecular weight was determined by the Signer method of isothermal distillation (Clark 1941). Twenty-five milligrams of free acids dissolved in methanol were equilibrated at 35°C with 9.9 milligrams of azobenzene (mol. wt. 182) in methanol. The average molecular weight of the free acids is 456 based upon the mean value for

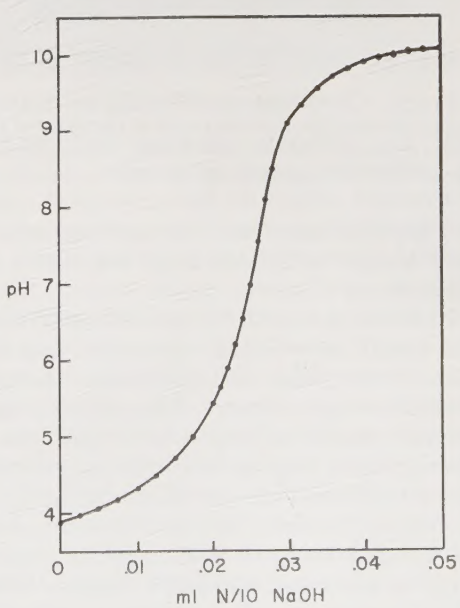

FIG. 5. Titration of 0.57 milligrams of the free acids with N/10 NaOH.

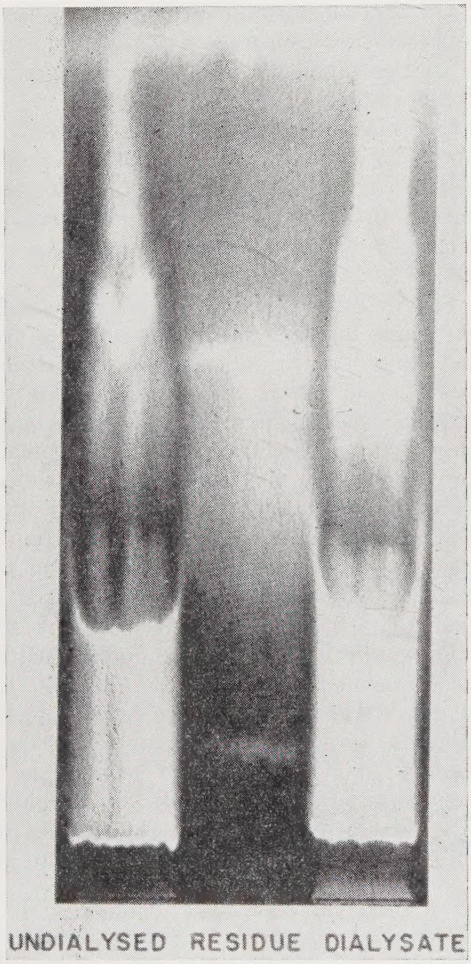

FIG. 6. Chromatograms of undialysed material and of the residue and dialysate of the salts of the acids extracted from lake water. Direction of solvent flow is from bottom to top.

two experiments. Since the average equivalent weight is 228 the acids are, again on the average, dibasic.

In connection with the problem of molecular size, I have found repeatedly that the salts of the acids are dialysable through Cellophane membranes. This is not a very refined criterion of molecular weight but it demonstrates that at least the salts are in true solution and are not colloidal (Fig. 6).

X-ray diffraction. X-ray diffraction studies using a layer of ethyl acetate extract dried on glass and utilizing a Geiger-Müller tube to measure the scattering indicated that the material was amorphous. A powder pattern gave substantially the same result.

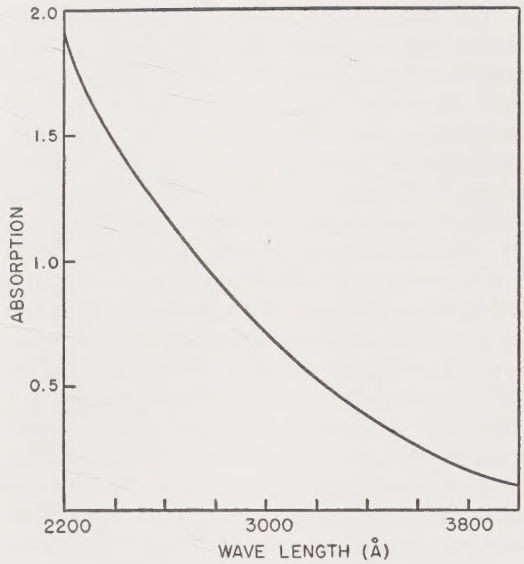

FIG. 7. Ultra-violet absorption spectrum of the salts of the acids extracted from lake water.

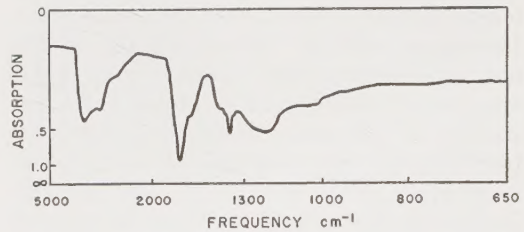

FIG. 8. Infra-red absorption spectrum of the free acids.

Ultra-violet absorption. Measurements of the ultra-violet absorption of the preparations were of little value in identification or as a method for following purification. As shown in Figure 7 the spectrum consists only of end absorption with no evidence of any peaks. The spectrum is not altered in acid or base or in organic solvents.

Infra-red absorption. In contrast to the ultra-violet, studies of the infra-red absorption spectra proved very useful. The spectra of several preparations are discussed below (see Bellamy 1954).

Free acids. Figure 8 is a reproduction of the infra-red absorption spectrum of an ethyl acetate extract. The band at 3340 cm^{-1} may usually be due to either an amine or alcohol group, but the latter is much more

likely judging from the small amount of nitrogen found on analysis. The normal frequency for unbonded hydroxyl is somewhat higher, and the lower figure is therefore probably due to hydrogen bonding. Absorption at 2860 cm^{-1} is due to alkane groups. The strong band at 1725 cm^{-1} may be due to carboxylic carbonyl or to the C=O of aldehydes or ketones. Proof that it represents a carboxyl group lies in the solubility of the material in base and the fact that the band is shifted toward 1600 cm^{-1} in the salt. This shift is characteristic of ionized carboxyl groups. The absorption near 1630 cm^{-1} may be caused by an aromatic structure, by unsaturation, or by partial ionization of the acid, while the shoulder at 1435 cm^{-1} and the maximum at 1380 cm^{-1} are probably due to alkane groups.

The spectrum indicates then that the sample is a mixture of carboxylic acids. The possibility of unsaturation or aromatic rings is not completely ruled out, but if these are present they are probably relatively unimportant. A large number of other fractions was analysed with similar results. No significant differences were ever observed between different chromatographic fractions.

Esters. The infra-red absorption spectrum of the methyl esters is shown in Figure 9. The carbonyl absorption has undergone a characteristic shift from 1725 cm^{-1} in the free acids to about 1740 cm^{-1} in the esters. The increased absorption at 2900 cm^{-1} and 1435 cm^{-1} is probably due to the increase in methyl groups (Jones and Cole 1952). The 1380 cm^{-1} band which was strong in the free acid spectrum is now much reduced, and the low band at 1605 cm^{-1} is alkene or aromatic. One point of significance is the strong absorption at 3350 cm^{-1}. This represents OH groups, but since the diazomethane used to form the ester reacts with all carboxylic hydroxyl, the conclusion is that OH groups are present in addition to those in the COOH. Attempts to acetylate the esters lead to the conclusion that the OH groups still present may be tertiary OH as these are usually difficult to acetylate.

Oxidation. Oxidation under various conditions was used in an attempt to determine the stability of the materials and if possible

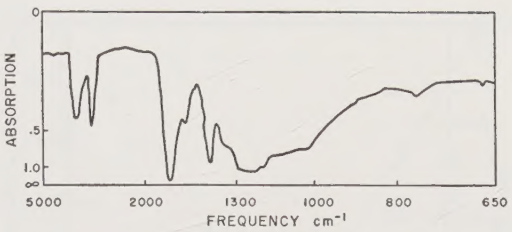

FIG. 9. Infra-red absorption spectrum of the methyl esters of the acids.

to identify some of the constituents. The results were as follows. Oxidation with $KMnO_4$ under acid conditions at room temperature will destroy all color within 30 seconds without appreciable effect on the fluorescence. Oxidation with $KMnO_4$ under alkaline conditions takes longer to destroy the color. Oxidation under either condition for a sufficient period leads to gradual loss of fluorescence, and subsequent extraction of the acidified solution with ether yields a colorless oily material which has only end absorption in the ultra-violet region. Its infra-red absorption spectrum is very much like that of the free acids. However the material will not react directly with ferric chloride as will the acids, but must first be converted to its hydroxamic acid derivative by treatment with thionyl chloride and alkaline hydroxylamine. This would suggest that oxidation has destroyed the phenolic or enolic portion of the material.

Quantitative studies

Weights of extracted materials. On two occasions sufficiently large quantities of water were extracted to enable reasonably accurate estimates of the amounts of materials. On April 2, 1956, 112 liters of Linsley Pond surface water were extracted with ethyl acetate in the manner described. Allowing for certain losses through handling, the value of 2.08 to 2.62 milligrams of free acids per liter was found. Assuming that about half of the material was either adsorbed onto the salts or was unextractable, there were between 4.16 and 5.24 milligrams per liter. A similar extraction of 160 liters on June 1, 1956, yielded a total of 334 milligrams. Since there was a possible loss of material equivalent to that in about 15 liters of water (by precipitation onto the walls of

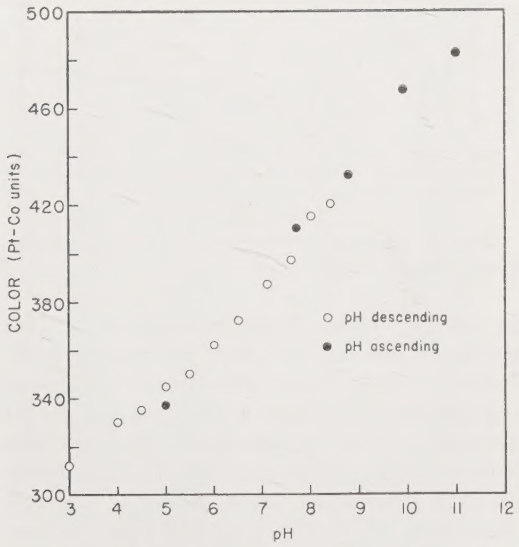

FIG. 10. The effect of pH on the color of the yellow materials isolated from lake water.

the still during evaporation) there were 2.09 to 2.30 milligrams of free acids per liter. Again assuming a 50% loss during extraction the figures would be 4.18 to 4.60 milligrams per liter. The mean of the two estimates is 4.5 milligrams per liter. It is interesting to compare this with the figure of 2.9 milligrams per liter for the sestonic organic matter in Linsley Pond (Deevey 1940).

It should be pointed out that this includes only the acidic materials in solution. Left behind were the bases, neutral, and amphoteric compounds. Thus the dissolved organic matter, at least in Linsley Pond, is considerably more than that in the seston.

If the quantity of acids present is judged from the color (see below) the figure for Linsley Pond is 8–9 milligrams per liter. Possibly the extraction procedure is less efficient than assumed.

Relation of color to pH. On several occasions it was noted that addition of acid to concentrates or extracts resulted in a lightening of the color, while addition of base had the opposite effect. Since the two previous investigations of this phenomenon (Ohle 1934, Hutchinson, in press) had resulted in somewhat different conclusions, this was a good opportunity to resolve the problem using relatively pure material.

Accordingly an experiment was carried out in the following manner. Six milligrams of free acids were dissolved in dilute NaOH solution to give a final pH of 8.4. The color was read in a Dubosq visual colorimeter using a platinum-cobalt standard solution with a color of 250. The pH was lowered in steps by the addition of dilute HCl and the color read at each step. At pH 3.0 the pH was gradually increased with dilute NaOH and a second series of readings was taken. The reagents were sufficiently concentrated to minimize dilution. The results plotted in Figure 10 show that the effect of pH on color is reversible and nearly linear. Tabular data reveal however that the effect is not completely linear, the per cent increase in color per unit of pH ranging from 3.0 to 8.0 with a mean over several trials of 6.7.

Relation of color to concentration. Once the relation between color and pH was established it was of interest to determine the relation between color and concentration. Using these two sets of data it should be possible to arrive at close estimates of the amounts of the colored materials in natural waters. Figure 11 shows the effect of adding various amounts of the free acids to distilled water. The color was determined as in the preceding section. The acid used had a carbon content of 53.35%.

Clearly Beer's law holds within the limits of the experiment. It is unfortunate that due to the limitations of the method of determining the color the experiments had to be stopped just above the point where James (1941) found significant deviations from linearity. As mentioned previously, according to these data Linsley Pond should contain about 9 mg/L of the free acids, but extraction gives a figure just about one-half as large even after allowing for losses. Probably the difference is due to the difficulty of estimating extraction efficiency.

Properties of biological interest

The properties discussed below are those likely to be of more significance to the biota than those already mentioned.

Effects on light. It is evident from the ultra-violet and visible absorption spectra that the yellow materials must play a very important role in determining the quantity and quality of light reaching various depths.

The work of Juday and Birge (1933) leaves little more to be said in this regard.

Buffering capacity and pH. One possible role of the materials in nature may be as a buffering agent. The shape of the titration curve (Fig. 5) shows that an acid lake containing a reasonable amount of coloring matter would tend to remain acid while a basic lake would remain basic.

In addition the acids may account for some of the acidity of bog lakes, since an aqueous solution of them may have a pH as low as 3.6.

Stability. Evidence for the stability, that is the resistance to destruction, of the materials comes from two sources. Oxidation by refluxing with acid permanganate for two hours destroyed the color and fluorescence but not the infra-red absorption spectrum. This indicates that at least part of the material may be difficult for organisms to utilize. It also means that caution must be exercised in assessing dissolved organic matter by wet oxidation methods.

The second line of evidence comes from allowing water to stand and comparing the final chromatographic pattern with the original. Two samples were so treated. Water from Upper Linsley Pond and Moodus Reservoir, Connecticut, was filtered, dried, extracted with 80% ethanol, and the extracts chromatogrammed using the pyridine solvent. Portions of the same samples were allowed to stand unfiltered and in clear glass bottles at room temperature and light conditions for 4½ months and then were treated similarly. The results are shown in Table 3. Aside from the loss of the same chromatographic zone in each case the patterns showed no significant change.

Effects on algal cultures. In view of the abundance of the acids in lake water it would be of very great interest to know whether they have any direct effect on the biota, using the word direct as opposed to indirect for such effects as may be brought about for example through alteration of light quality. Experiments were performed to obtain data on this matter.

Unialgal bacteria-free cultures of *Haematococcus pluvialis* were grown in liquid mineral culture at pH 7.2. After growing through one transfer without the soil extract

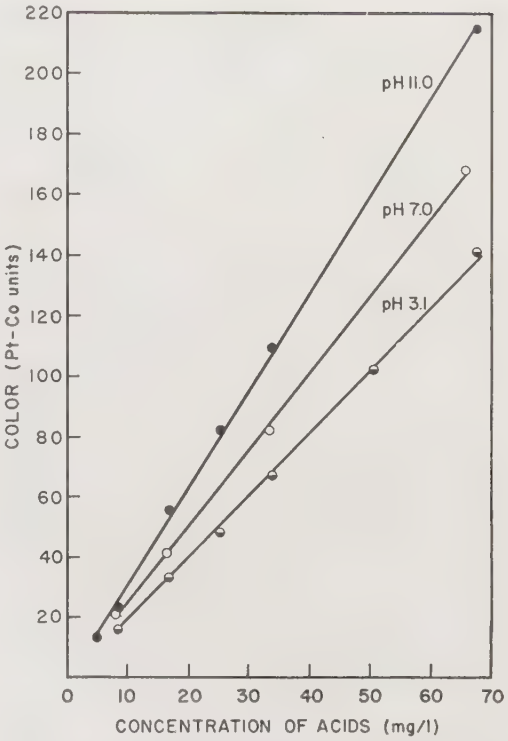

FIG. 11. The relation between the color and the concentration of the acids extracted from lake water as determined by adding various amounts of the free acids to distilled water.

TABLE 3. *Chromatographic zones of 80% ethanol extracts of two samples of lake water, extracted at the time of collection (April 3) and after standing untreated for 4½ months (August 18)*

Upper Linsley Pond		Moodus Reservoir	
April 3	August 18	April 3	August 18
00	00	00	00
.13		.16	
.25	.22	.24	.25
.50	.50	.56	.66
.76	.81	.80	.84

usually added (see, for example, Pringsheim 1946), but with added trace elements, four cultures were set up in duplicate, adjusted to pH 7.2, and allowed to grow for six days. The results are shown in Table 4. Apparently both the soil extract and 50 mg/L concentration of acids stimulated growth. There is no way to measure the relative effectiveness of each however, as soil extract cannot be quantified.

TABLE 4. *Effects of free acids and soil extract on the growth of* Hematococcus pluvialis

Material added to medium	Cell count
None	53
5 mg/L of free acids	38
50 mg/L of free acids	279
Soil extract	110

TABLE 5. *Effects of free acids and soil extract on the growth of* Chlamydomonas Reinhardi *and* Scenedesmus quadricauda

Material added to medium	Cell count	
	Chlamydomonas	Scenedesmus
None	54	650
1 mg/L of free acids	58	950
5 mg/L of free acids	57	1,150
50 mg/L of free acids	67	22,100
Soil extract	64	87,000

Hematococcus pluvialis does not normally occur in lakes, and therefore more pertinent data were sought using unialgal bacteria-free cultures of *Scenedesmus quadricauda* and *Chlamydomonas Reinhardi*. All of the cultures were supplied by Dr. Vernon W. Proctor whose suggestions and assistance were invaluable during this part of the investigation. Before being used the organisms were grown through two successive transfers without soil extract but with trace elements. The cultures were begun at pH 7.2, the *Chlamydomonas* being counted after five days and the *Scenedesmus* after eleven days. The results shown in Table 5 were surprising insofar as the *Chlamydomonas* cultures were concerned, since the cultures containing soil extract and those with 50 mg/L of free acids had looked much greener and healthier than the rest. In the case of *Scenedesmus* the soil extract had the greater effect as opposed to the situation in *Hematococcus*. This is undoubtedly due to the fact mentioned, that it is impossible to measure the concentration of soil extract. The yellow materials are effective at some concentration between 5 and 50 mg/L. No attempt was made to determine whether any of the material was used up.

Relation to iron. Iron is essential to phytoplankton. It is however almost insoluble at the pH values normally encountered in lakes. Cooper (1937) has shown for example that at pH 7.0 not more than 4×10^{-8} mg/L of iron can be present in solution. Some algae are capable of utilizing iron as ferric oxide (Goldberg 1952), but others undoubtedly require the iron to be in solution. Fortunately for these latter forms much iron is in solution in lakes as, for example, in Linsley Pond where Hutchinson (1941) has reported 0.03 milligrams per liter. The question is then, how is the iron held in solution? Some preliminary experiments with the yellow acids look encouraging.

In one experiment a solution of 10 mg of the sodium salts of the acids in 35 ml of distilled water was treated at pH 6.0 with 0.5 mg iron as $FeCL_3$. The molar ratio of acids (mol. wt. 456) to iron was 2×10^{-5} to 1×10^{-5}. The pH was raised stepwise with NaOH to 13.2 with frequent filtering (Millipore), but no precipitate was observed until the solution had stood overnight. A control of distilled water containing the same amount of iron but no yellow material precipitated completely on being brought to pH 5.8.

A second experiment used acids and iron in the molar ratio of 4×10^{-5} to 1×10^{-5}. Again distilled water was used as a control and both solutions were adjusted to pH 9.5. Half of each sample was allowed to stand under conditions of room light and temperature, and the other half was dialyzed against distilled water at pH 9.5. The iron in the control precipitated rapidly leaving hardly a trace in solution. The iron in the other sample had not settled out after four weeks and still gave a strongly positive test with 1,1′ dipyridyl after reduction with bisulfite. Of the two samples which were dialysed for four weeks, the control dialysate gave a negative test with dipyridyl, and the dialysate of the sample containing the acids, although yellow in color, also gave a negative test. It was noted that the rate of passage of the yellow material through the membrane seemed to be slower than usual.

Distribution

Very little work has been done on the temporal or spatial distribution of the coloring matter within Linsley Pond. Chromatograms of surface and ten-meter sam-

ples were similar, but this was after the fall stratification had disappeared. No data are available during periods of stratification except that on several occasions water from ten meters was much yellower than surface water.

However considerable data are available regarding different lakes. On April 3, 1955, chromatograms (pyridine solvent) of 80% ethanol extracts of surface waters of five Connecticut lakes had the chromatographic zones listed in Table 6. Eight months later, on December 3, 1955, a surface sample of Lower Linsley Pond water had the following pattern: 00, .17, .24, .57, .78, .83. Again the zone at R_F .83 was yellow but did not fluoresce.

The results of a more extensive series of collections on July 8, 1956, are shown in Table 7 and Figure 12. Included in the table are values for color (platinum-cobalt scale and at pH 11) and bicarbonate. The most notable feature is that all of the lakes have very similar patterns. The fact that some show two zones between R_F .70 and .76 while others show only one is due to the difficulty of distinguishing these weak zones. It is interesting that the two lakes having zones with R_F .94 are Dooley Pond and Lower Linsley Pond. These are the same two lakes which had non-fluorescing zones on the previous occasion. The non-fluorescing zone did not appear in the later series.

Finally, an 80% ethanol extract of a sample of Long Island Sound water collected on April 3, 1955, showed three yellow fluorescent zones. No R_F measurements were possible due to overloading of the chromatogram with salts.

Table 6. *R_F values of chromatographic zones of 80% ethanol extracts of five Connecticut lake waters collected on April 3, 1955*

Upper Linsley Pond (eu-dystrophic)	Lower Linsley Pond (eutrophic)	Dooley Pond (very eutrophic)	Moodus Reservoir (eutrophic)	Bashan Lake (oligotrophic)
00	00	00	00	00
.13	.14	.14	.16	.15
.25	.25	.25	.24	.25
.50	.48	.54	.56	.55
.76	.73	.75	.80	.78
	.81[1]	.81[1]		

[1] Yellow but not fluorescent.

Table 7. *Color and bicarbonate values and R_F values of chromatographic zones of 80% ethanol extracts of eleven lake waters collected on July 8, 1956*

Lake	Color at pH 11	Bicarbonate ppm[1]	R_F						
			A	B	C	D	E	F	G
Bashan	5	9	00	.13	.13–.39	.43		.76	
Quassapaug	10	11	00	.13	.13–.32	.48		.74	
Queechy (N. Y.)	11[1]	123	00	.13	.13–.35	.41		.74	
Mendota (Wis.)	12	—	00	.11	.11–.31	.43		.73	
Quonnipaug	15	33	00	.12	.12–.31	.46		.74	
Dooley	17	37	00	.11	.11–.36	.36		.72	.94
Zoar	23	123	00	.11	.11–.36	.41	.70	.74	
Columbia	25	14	00	.11	.11–.36	.38	.71	.75	
Lower Linsley	28	63	00	.11	.11–.34	.34	.71		.94
Bantam	32	35	00	.12	.12–.29	.51		.74	
Moodus	50	15	00	.12	.12–.32	.46	.73	.76	

[1] Figures taken from Deevey 1940. Color adjusted to pH 11. All but Queechy and Mendota are Connecticut lakes.

True nature of the fractions

It is possible that the large number of chromatographic zones really represents an equal number of different compounds. However in view of the fact that no differences are observable between their ultraviolet or infra-red spectra it is more likely that the different zones represent variants of a single compound or small number of related compounds. For example if the acids are dibasic and have, as seems to be the case, several hydroxyl groups, a very large number of lactones or lactides is possible, each of which would presumably differ chromatographically from the rest.

An alternate explanation is that the different zones are different salts. In order to test this hypothesis ether solutions of the free acids were shaken with various salt solutions, and the aqueous layers were chromatogrammed using the pyridine solvent. The results are shown in Figure 13 and Table 8. Several facts are immediately apparent. The fluorescent zone at the origin, R_F 00, is associated with Ca and Mg although when mixed with Na_2CO_3 it disappears (last two rows). This zone is probably equivalent to Zone A in Table 7. The second zone, R_F .07–.11, appears in the presence of Na, K, or Rb and can be rec-

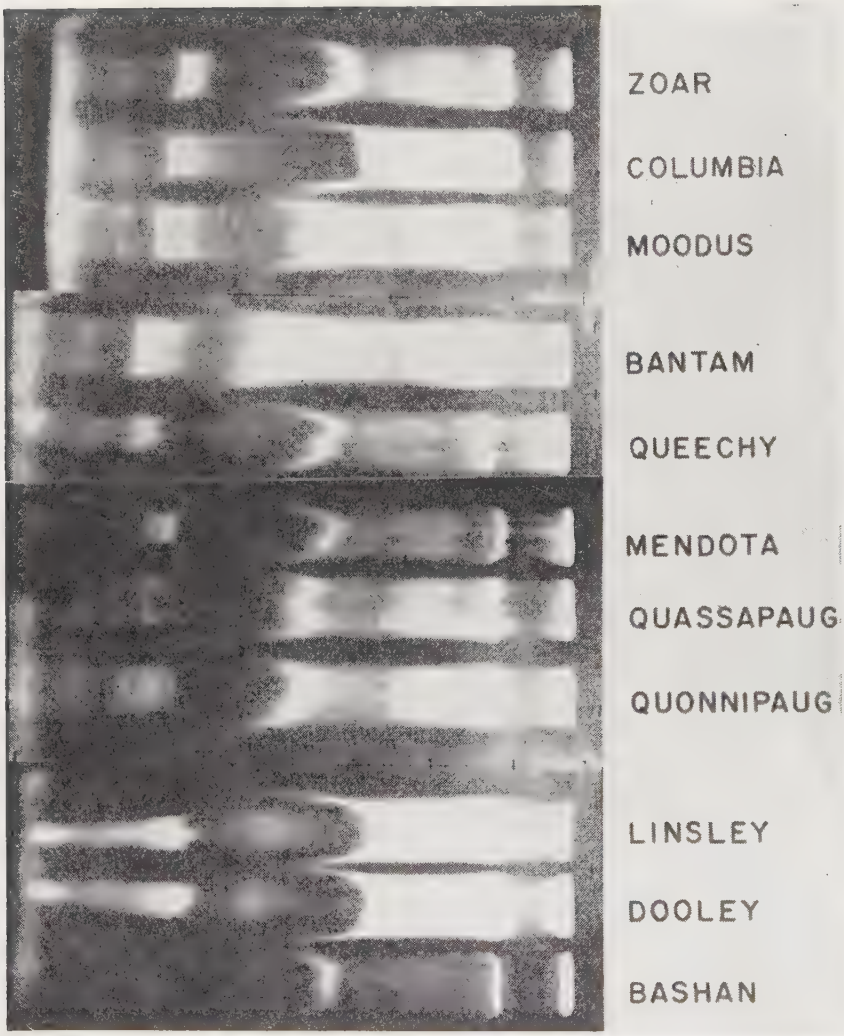

FIG. 12. Paper chromatograms of 80% ethanol extracts of nine Connecticut lakes, one New York lake, and one lake in Wsconsin. Direction of solvent flow is from right to left.

ognized by the fact that it is composed of two very close zones, the faster of which is yellow and the slower, orange. The same appearance belongs to the third zone, R_F .14–.18, in the case of the NH_4OH treatment, and to Zone B in Table 7. It is likely that these zones are equivalent. It is of some interest that the other monovalent cation Li did not produce the same result. The zone R_F .14–.18 in the other two cases does not seem to be represented in Table 7. The broad zone, R_F .16–.30, found only after mixed anion treatment may be equivalent to Zone C in Table 7. The other zones are too indefinite to allow interpretation.

Thus it has been possible to "synthesize" several of the chromatographic zones normally found in lake water. Further evidence for the salt hypothesis results from the effects of electrolytic desalting (Consden *et al.* 1947). Such treatment results in marked changes in the original chromatographic pattern.

Very few zones are listed under K_2CO_3 in Table 8, while in Table 2 it is seen that a large number of zones resulted from extraction into potassium bicarbonate. The reason probably lies in the fact that in the latter case considerably larger quantities of material were involved in the chromatography,

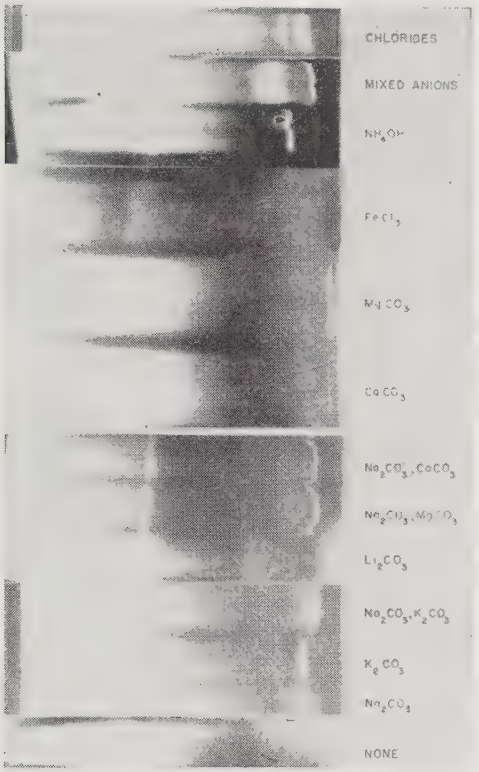

FIG. 13. Paper chromatograms of the aqueous layers resulting from shaking ether solutions of the free acids with various salt solutions. Direction of solvent flow is from right to left. "Chlorides" refers to a solution containing equal parts of the chlorides of Ca, Mg, Na, K, and Fe plus a small amount of NH_4OH to permit extraction. "Mixed anions" refers to a solution containing equal parts of $CaNO_3$, $MgSO_4$, Na_2CO_3, $NaHCO_3$, KCl, and $FeCl_3$.

and thus components present in lower concentration would become obvious. This does not alter the hypothesis that the strongest zone is connected with the presence of potassium, for under the circumstances, and particularly if there is a tendency toward an equilibrium mixture of salts the other zones could be due to cations present other than K.

Origin

The origin of the yellow materials has not been fully investigated, but some data are available. The seston collected from thirty liters of Linsley Pond surface water during a bloom of *Anabaena* was dried and extracted with 80% ethanol. Subsequent

TABLE 8. *Chromatographic zones of the aqueous layers resulting from shaking ether solutions of the free acids with various salt solutions*

Solution	R_F						
	00	.07–.11[3]	.14–.18	.16–.30	.49–.50	.54–.63	.71–.77
Water						×	×
NH_4OH			×[3]			×	×
K_2CO_3		×				×	
Na_2CO_3		×				×	
$CaCO_3$	×					×	
$MgCO_3$	×					×	
$LiCO_3$						×	
RbCl + NH_4OH		×				×	?
$FeCl_3$	abs.[4]					×	
Mixed anions[1]	×	×	×	×	×	×	×
All chlorides[2] + NH_4OH	×	×	×			×	×
Na_2CO_3 + K_2CO_3		×				×	
Na_2CO_3 + $MgCO_3$		×				×	
Na_2CO_3 + $CaCO_3$		×				×	

[1] Consisted of equal amounts of $CaNO_3$, $MgSO_4$, Na_2CO_3, $NaHCO_3$, KCl, and $FeCl_3$.

[2] Contained equal parts of $CaCl_2$, $MgCl_2$, NaCl, KCl, and $FeCl_3$, plus a small amount of NH_4OH to permit extraction into the aqueous phase.

[3] This zone has a yellow front edge and an orange trailing edge.

[4] Absorbs ultra-violet light without fluorescing.

acidification of the aqueous solution and extraction with ethyl acetate yielded no color. It has been shown however that the bottom mud of Linsley Pond contains large amounts of yellow materials which react toward ion exchange in the same way as the coloring matter of the water.

Extracts of sandy and black soils from the drainage area of Linsley Pond were made by soaking the soils in distilled water. Such extracts are yellow, fluoresce, and behave on chromatograms similarly to lake water extracts. Part of the color can be extracted from acid solution by ethyl acetate, and the dried material resembles the free acid preparations. The soil extracts are reversibly lightened with acid. In addition the ethyl acetate extracts of the black and sandy soils had essentially the same infrared spectrum as the free acids. It should be pointed out however that this similarity

may be misleading, as almost any mixture of carboxylic acids would have a reasonably similar spectrum.

DISCUSSION

This investigation of the yellow substances found in lake water has weighed certain earlier suggestions as to their nature and role and has extended our knowledge of them.

The acidic nature of the materials is now firmly established, not only through their solubility relationships as in the work of Aschan (1908, 1932), but by ion exchange, infra-red analysis, and the participation in reactions possible only through mediation of the carboxyl group. It is of interest that all of the coloring matter in Linsley Pond can be assigned to this class of compounds.

The values for per cent carbon and hydrogen are slightly higher than those cited by Aschan, while those for oxygen and nitrogen are lower. No tests were made for phosphorus, but sulfur and the halogens are absent, and it appears on the basis of the molecular weight and infra-red analyses that the nitrogen is not part of the colored material.

The molecular weight of 456 is exactly twice the equivalent weight, indicating the presence of two carboxyl groups. Infra-red analyses of the methyl esters show that additional hydroxyl groups are present, and acetylation experiments suggest that some of these hydroxyl groups may be tertiary. In any event these groups probably participate in hydrogen bonding. Several reactions indicate the presence of enols or phenols, but the question of aromaticity has not been resolved. The equivocal nature of the results may mean that if aromatic structures are present they are of relatively minor importance. Similarly it is not known for certain if the material is unsaturated, but the weight of the evidence does favor the existence of double bonds. In connection with aromaticity it may be of significance that the materials possess a delicate fruity odor.

Most investigators have hitherto believed the yellow materials to be colloidal (Ohle 1940, James 1941), but it is clear from the results of the dialysis experiments (Fig. 6) that such is not the case. The previous results were probably due to colorless colloids.

The spectral characteristics of the isolated materials bear out the results of studies on light penetration in lakes by Juday and Birge (1933), and the visible absorption curve of the isolated substances is very similar to the recently published curves by Skopintsev and Krylova (1955) and Gorham (1957) for light absorption by lake water. In addition the intense absorption at the shorter wavelengths is reminiscent of the filter-passing materials found in sea water by Clarke and James (1939) and Burt (1953). Absorption in the ultra-violet results in fluorescence, a phenomenon noted for lakes by Merker (1931), but ascribed by him to excretory products of organisms.

The materials are highly resistant to chemical oxidation and are apparently rather stable toward biological decomposition. This raises the question as to whether they are identical with the materials postulated by Krogh (1931) and Krogh and Lange (1931) and most recently studied by Skopintsev (1949). Experiments are being planned to test directly the availability of the carbon of the yellow substances to the microorganisms found in lakes.

The effect of pH on color agrees with the results of Hutchinson (in press) rather than with those of Ohle (1934) since no sharp break was found. It is interesting that Hutchinson, working with water from Jones Lake, a highly colored Carolina bay, found about the same increase in color between pH 3 and pH 11 as in this investigation. However, Gorham (1957) recently found no such significant change in color with pH of two eastern Canadian lakes. The reason for this discrepancy is not known but in any event it is suggested that measurements of color be made in the future only after adjusting the pH to a standard value, preferably on the alkaline side for increased accuracy.

The buffering capacity of the materials is evident from the shape of the titration curve (Fig. 5), but the extent to which the substances act as buffers in nature is not known. It is probable though that their effect is much more important in a lake such

as Moodus Reservoir where the color/bicarbonate ratio is about 3.3 than in Queechy Lake where the ratio is 0.09.

The reasons for the apparently large number of yellow compounds need further study. The number of yellow acids has not yet been determined, and it is possible that several acids or several acids and their isomers are responsible for some of the multiplicity. However, it is probable that the salt hypothesis will prove at least partially correct. It has not been possible to synthesize completely the chromatographic pattern of normal lake water, but this is likely due to uncertainty as to all of the factors involved. Studies are planned which will involve elution and study by flame photometry of individual zones.

The fact that the yellow materials have essentially the same chromatographic pattern at different times and in widely different lakes is of great interest. Even more interesting is the fact that Lower Linsley Pond and Dooley Pond differed on two occasions from all of the other lakes. These two are the most eutrophic of the lakes studied, but further correlations are not available at present. With regard to the similarity of the materials from different lakes, although it was found during this investigation that anion exchange resins were highly efficient in removing the substances from the water, Nydahl (1951) and Westland and Langford (1956) have seemingly found the efficiency to be much less. The significance of this discrepancy is unknown.

Although Kalle (1949) and Johnston (1955) have found yellow fluorescent substances in sea water it is reasonably certain (partly on the basis of the color of the emitted light) that these substances are not the same as those described in this paper, but the materials found in Long Island Sound (see section on distribution, above) are probably so.

As previously mentioned the quantity of acidic material in Lower Linsley Pond surface water is probably between 4.5 and 9.0 milligrams per liter. However, it has been shown that colorless acids are present along with the yellow acids. One way in which an estimate of the relative proportions of the two can be found is by comparing the data with those of Juday and Birge. Using their data Hutchinson (in press) has shown that the water color is essentially constant at 6.6 platinum-cobalt units per milligram of allochthonous soluble organic matter in lakes of differing carbon content. Assuming that this allochthonous organic matter is all colored, and comparing this figure with that of 2.5 obtained with the isolated material (Fig. 11, pH 7.0), the latter is approximately forty per cent colored. One suggestion as to the nature of the remaining colorless sixty per cent is that at least part of it may consist of similar material which has been bleached by sunlight (Whipple 1927).

Regardless of the problem of color the fact remains that the water contains reasonably large quantities of materials in solution which have hitherto been classified as "proteins" and "carbohydrates," neither of which is correct. They have probably not been included in the ether extracts of other workers because of the fact that they are not extractable from basic solution.

The nature of the growth-stimulating action of the materials is not known. It is not likely to be through their acting as nutrients, for in the experiments in which low concentrations failed to exert any effect the materials were still apparently present after a few days. The possibility of trace-element stimulation cannot be ruled out, but trace elements were added and no attempt was made to use specially purified reagents in the medium. In this regard it may be pointed out that although the large quantities of acid required to produce a stimulatory effect compared to the quantities estimated to be present in nature at first suggest that the action may really be due to impurities, algae grown in culture almost always require higher concentrations of nutrients than they do under natural conditions. Therefore it is just as likely that the high concentration of acids required is due to a peculiarity of culturing rather than to an impurity. An alternate explanation of the stimulatory effect lies in the behavior of the materials toward iron. As de Kock (1955) and many others have noted, plants grow very much better when they are supplied with a mobile form of iron in the form

of a soluble complex or chelate. Pringsheim (1946) suggests that the role of the soil in his soil-water cultures is of this nature, and it may be of significance that of the three algae used here, only those two normally requiring soil extract were stimulated by the yellow substances. It still remains to be determined however whether the iron which is held in solution (or suspension) by the acids is available. The results of the dialysis experiment suggest that the iron may be in a peptized state (Zaĭdes 1935), or alternatively that the complex formed is of too large a size to dialyze. Experiments are now in progress to test directly the availability of the iron. Finally, one other possibility which must be considered is that the materials act by chelating not only desirable elements but by reacting with other substances normally inimical to growth.

The similarities between the yellow materials of the soil extracts and those isolated from lake water leave little doubt that the latter do originate in the soil. There is some evidence that they originate in the lake sediments as well, and it is probable that they will be found wherever vegetation decomposes. The question arises as to whether the dissolved substances are the same as soil "humic" acids(Odén, 1922), for although they have been called such this has been purely a matter of convenience. There is general agreement that soil humic acids are aromatic and have a molecular weight of about 1400, and on this basis the two are different, but the problem is complicated by the demonstration that the acids here obtained from the soil extracts are apparently not predominately aromatic. This coupled with the general disagreement concerning the other aspects of the nature of humic acids renders comparison unprofitable. Therefore I feel that if the dissolved substances must have a name, the privisional term *humolimnic acids* is appropriate, for while recognizing an undoubted relation to the humic acids it implies that the materials in lake water are not necessarily identical with them.

Finally, it is not likely that any significant fraction of the materials described represents the "ectocrines" envisaged by some investigators as playing a role in succession in lakes. Although a small portion (probably colorless) may derive from living organisms and so vary with the dominant species, most of it seems to be present as a constant factor over long periods of time. In other words the materials are probably one of the conservative features of the limnetic environment and as such their effects within the lake are likely to be more subtle and extend over longer periods of time rather than to mediate rapid changes.

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The Feeding Behavior of the Seastar *Evasterias troschelii* Stimpson

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ABSTRACT

Kymograph recordings of shell movements of 4.0–5.5 cm long clams (*Protothaca staminea*) during attacks by the seastar (*Evasterias troschelii*) showed: (1) in 21 experiments the valves were pulled apart at a mean rate of about 1.0 mm per minute, as measured at the mid-ventral edge of the clams; (2) clams constantly tried to close during the initial opening of them by the seastars and then at longer intervals thereafter for a period usually not exceeding 4 hours.

A specially constructed, false lucite "clam" containing *Mytilus* meat as bait was opened by *Evasterias* against known resistances applied to the approximate center of the upper, free-moving valve of the "clam." Kymograph recordings of shell movements of this "clam" during seastar attacks showed: (1) the curves showing the openings agree closely with those showing openings of live clams; (2) an average sized *Evasterias* may exert forces in excess of 5000 grams; (3) having lifted such weights, the seastar may hold them lifted for several hours; (4) in 14 experiments, weights varying from 700 to 5500 grams were lifted at an average speed of about 0.8 mm per minute, equal to an increase in gape of about 1.2 mm per minute during the lift. Maximum lifts varied from 2.8 to 13.5 mm with a mean of 6.6 mm.

Experiments and observations showed that only the longitudinal musculature of the tube feet is responsible for the forces exerted by the seastar in opening bivalves. However, there are strong indications that the seastar is capable of "locking" itself in a rigid state before commencing the pull.

If prevented from applying force, *Evasterias* will still succeed in feeding on bivalves, but the digestion period is considerably prolonged due to difficulties in inserting all lobes of the stomach into the prey.

INTRODUCTION

O. F. Müller (1806) was the first to note the eversion of the stomach in seastars. Eudes-Deslongchamps (1826) observed the same phenomenon and realized that to feed on bivalves in this manner, the seastar would first have to open its prey against the force of the adductor muscles. He suggested that *Asterias* secretes a poison which eventually relaxes these muscles. Since then the method by which seastars of the *Asterias* type open their prey has been the subject of much research and even more theorizing. The poison theory was accepted among others by Forbes (1841) and Hamann (1885).

In the meantime De Montauge (1879) introduced a new theory, stating that seastars with extraoral digestion simply starve or suffocate their prey by enveloping them.

The first paper based on solid experiments was published by Schiemenz (1896). He reviewed the literature and rejected all theories advanced. His experiments and observations led him to conclude that *Marthasterias glacialis* opened its prey by force. Möbius (1877) had first observed the humped-up feeding position of *Asterias rubens*, and Hamann (1885) had noted that this seastar always turned the hinge of its bivalve prey away from the mouth opening prior to feeding on it. Schiemenz now interpreted the humped-up position as necessary for the seastar to pull with its tube feet on both valves of its prey at the same time. Schiemenz realized that merely asserting that the pull was accomplished by the tube feet is not a sufficient explanation but states, "Ich will mir hier nicht weiter auf physikalischer Betrachtungen einlassen." It is Mead (1899) who in accepting Schiemenz's theory goes a step further and states that the seastar displays a tendency to

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straighten the arms, resulting in a steady pull on the shells. MacBride (1906) states that *Asterias* uses its tube feet alternately, enabling it to pull for a long time and to overcome eventually the bivalve adductor muscles. Modifications of Mead's explanation have often appeared, thus by Miner (1950) and Clark (1956) who state that the opening is accomplished by contraction of the muscles of the middle parts of the arms. None of these explanations has been generally accepted. Korringa (1952) sums up the opinion of many observers in stating, "the poor supporting skeletal structure in the starfish renders it doubtful whether its steady pull is really so powerful."

The poison theory reappeared when Van der Heyde (1922) found that the stomach secretion of *Asterias rubens* exhibited toxic effects. Ten years later, Sawano and Mitsugi (1932) obtained toxic effects on the hearts of oysters by stomach extracts from several species of Japanese seastars. Recently Aldrich (1954) demonstrated a toxic effect of extracts of the pyloric diverticulum of *Asterias forbesi* on several species of bivalves. However, no one has isolated the supposedly toxic substance. A toxic theory has also been supported on other grounds. Fasmer (1950) found at Vancouver that *Pisaster ochraceus* digested oysters through small meshed screens of cages containing these bivalves, and rightly concluded that no mechanical means could have been employed to open them. Reese (1942) criticized Schiemenz's theory on the basis of experiments demonstrating the strength of the adductors of oysters and clams, finding it unbelievable that seastars could overcome them. Korringa (1951) assumes that the seastars paralyze their prey "when the mollusc—embraced by the starfish—opens a bit to find out whether the danger is still there."

A combination of the mechanical and the toxic theory was first advanced by Blegvad (1914) who apparently visualized a simultaneous action of both. Mortensen (1927) and Lee (1951) are more reserved on the toxic action, but concede that if any toxic substance is present, it is probably used only after an initial opening by force. Imai (in Cahn 1950, and personal communication) holds the opposite view. He states that *Asterias amurensis* begins its attack on an oyster by pouring poison into the water in front of the oyster's inhalant siphon. This stimulates the oyster to close its shells at once but enough poison has already entered to relax the adductor muscle. Then the seastar completes the opening by its tube feet.

Meanwhile Schiemenz's theory never lacked support (Coe 1912, Hancock 1955, and others, besides numerous textbooks). Thus Young (1926) observed *Coscinasterias calamarias* opening brachiopods by force.

However, Schiemenz experimental setup for proving that *Marthasterias* can open bivalves by mechanical means is open to the just criticism that it shows only that the seastar can hold on to a clam with its tube feet against a certain force which, as pointed out by Blegvad (1914), was exerted only momentarily.

It remained for Feder (1955) to establish beyond any doubt that seastars can open their prey by force alone. He cut the adductors of mussels which were then tightly bound by rubber bands and observed *Pisaster ochraceus* open them against the force of the bands. By measuring the amount of gape and finding how many grams were necessary to duplicate it, he found that a *Pisaster* of about 30 cm in diameter could exert forces up to 4000 g. However, Feder concludes that the feeding of *Pisaster* on bivalves cannot be explained in terms of one process alone. He made the important observation that *Pisaster* could insert its stomach through openings of as little as 0.1 mm in width and that even bivalves with supposedly tightclosing valves nearly always had permanent openings exceeding this measurement. Thus, the seastar would not necessarily have to open its prey at all to feed on it. He found that opening by force is especially used on smaller prey, while in the case of large *Mytilus*, for example, the seastar would generally simply insert its stomach through the permanent opening of the byssus region. He also states that some bivalves show a remarkable lack of sensitivity and response, even to the stomach of a seastar attacking it, and that this

factor may also be of importance in the feeding of *Pisaster* on such prey.

During the preparation of this manuscript, Lavoi (1956) has published a work on the feeding behavior of *Asterias forbesi*. While his methods differ from those of Feder (1955) his results are essentially similar.

The present studies were undertaken to throw further light on some of the problems linked with the old seastar-bivalve question, as discussed above. They could not have been undertaken without the generous support and help rendered by several persons. The director of the Department of Oceanography and the Friday Harbor Laboratories of the University of Washington, Dr. R. H. Fleming provided all the research facilities as well as special equipment as the need arose. Dr. Dixie Lee Ray of the Department of Zoology, University of Washington, was a constant inspiration to the author and assisted in the collection of nearly all the experimental animals. Mr. Marcel Wenneken of the Department of Oceanography, University of Washington, and Dr. Frank Gwilliams of the Department of Zoology, University of California at Berkeley, gave invaluable technical advice on the use of different kinds of equipment. To all these persons the author wishes to extend his sincere thanks.

The author is also sincerely grateful to Dr. H. Feder of Hopkins Marine Station who freely communicated on the progress and results of his studies on *Pisaster ochraceus* long before they were published. He also gave valuable criticism on the results of the present work and permitted the author to publish and comment on some of his unpublished results.

For invaluable constructive criticism of the manuscript and the transformation of the same into understandable English, the author wishes to express his sincere thanks to Dr. H. H. Haskin of the Department of Zoology, Rutgers University.

The author also wishes to extend his grateful thanks to Dr. T. C. Nelson of Rutgers University and Dr. G. Thorson of the University of Copenhagen without whose support he would never have had the opportunity to do the present work.

Financial support from the Fulbright Foundation and the Danish State Research Foundation is gratefully acknowledged.

MATERIALS AND METHODS

The present studies were carried out at the University of Washington, partly in the Department of Oceanography and partly at the Friday Harbor Laboratories from December 16, 1954, to May 25, 1955. Both laboratories are equipped with excellent running sea-water systems, which makes it possible to keep experimental animals alive and vigorous for long periods of time.

Experiments and observations were done with *Evasterias troschelii*, with the clam *Protothaka staminea* and the mussels *Mytilus edulis* and *M. californianus* used as food animals. Both the seastars and the bivalves were collected in Puget Sound. *Protothaka staminea* is a sturdy clam with apparently tightly closing valves, but whether it has a small permanent opening was not determined.

Essentially four types of experiments were done and these are considered in sections as follows: I. Kymograph recording of movements of bivalve shells throughout a seastar attack; II. Kymograph recording of movements of valve of a false lucite "clam" opened by seastars against known resistance; III. Observations of seastars feeding through a screen; IV. Feeding by seastar with stomach contact only.

Methods and results with some discussion are presented separately for these four sections. Then follows a section on the method employed by *Evasterias* in opening bivalves by force, and finally a section dealing with other observations and a general discussion of the extraoral method of feeding.

I. KYMOGRAPH RECORDING OF MOVEMENTS OF BIVALVE SHELLS THROUGHOUT A SEASTAR ATTACK

Methods

Protothaka were used for these experiments. As shown in Figure 1, a small hole was drilled near the midventral edge of both valves of the clam (A) which was then firmly attached by one valve to a masonite plate

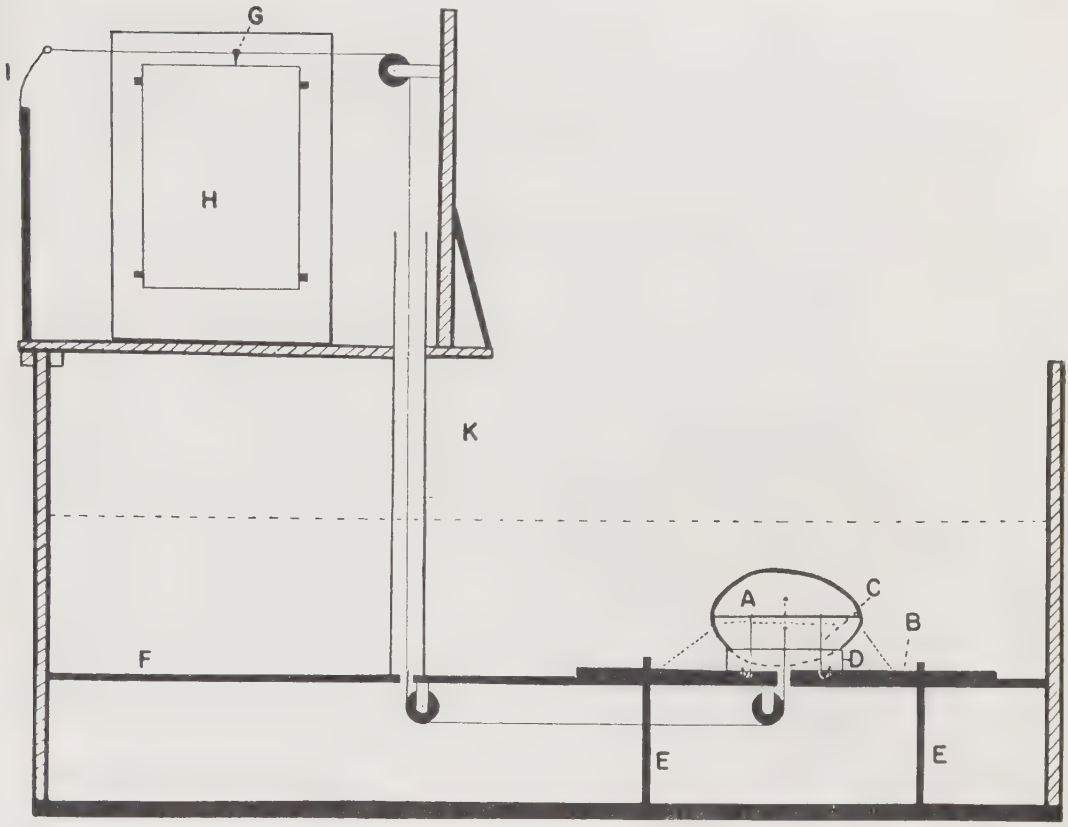

FIG. 1. Experimental setup for recording movements of bivalve shell during seastar attack (cf. text).

(B). The edges of the valves were filed at four places to insert four hooks (C) of stainless steel wire over the edge of the lower valve, and the hooks were then firmly attached to the plate. To prevent the seastars from wriggling the clam out of position the latter was first placed on a ring of lucite tubing (D), and after attachment of the hooks, the ring and hooks were covered with a layer of beeswax. (Fig. 2 shows a digested clam mounted in this manner.) By means of two holes fitting over two firmly attached lucite rods (E), the plates could be placed in an exact position on the false bottom (F) of the tank. A braided nylon line led from the upper, free-moving valve down through the holes of the lower valve, the plate and the false bottom, and over three specially-made, stainless steel pulley wheels to the horizontally-moving pen (G) of the ink-writer kymograph (H). To keep the line taut at all times another piece of nylon led from the pen to a weak return-spring (I). The nylon line could not be interfered with by the seastars as it was protected by the beeswax underneath the clam and by a glass tube (K) where it again emerged over the false bottom.

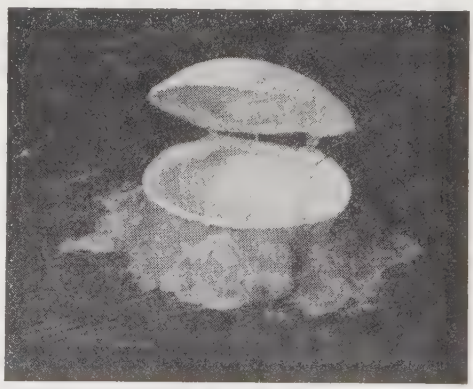

FIG. 2. Mounted clam shown after digestion of it by a seastar. Note the nylon line and the four hooks inserted over the lower valve.

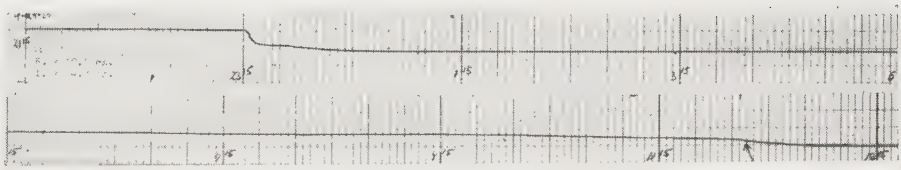

FIG. 3. Kymograph record of movements of bivalve shell during seastar attack. R = length of longest arm of seastar. l = length of *Protothaca*. Distance between vertical lines equals 4 minutes of recording. Distance between horizontal lines is 6 mm. True gape of clam is equal to recorded gape multiplied by 1.9 (cf. text).

As the nylon line had to be attached on the one-armed lever further from the fulcrum than the pen, movements of the valves were recorded on a decreased scale, the conversion factor to the true amplitude being 1.9. This factor and the thickness of the pen tended to obscure very small movements of the valves. Possibly the elasticity of the nylon line may have had the same influence, although only a very slight strain was put on it.

After mounting, the clams were kept on the plates in separate tanks for at least 24 hours to insure that they had recovered from the mounting. Figure 1 shows only one clam in position, while in the actual experiments three clams could be placed in the tank at the same time with the movements of the valves recorded on the same kymograph.

Towards the end of these studies a modified setup was used to permit the recording of slight shell movements by amplification of the recording and by substituting a thin stainless steel wire for the nylon line. Clams were mounted as described above; the wire was run from the upper valve down through a small hole in the bottom of the tank and connected with a weak, coiled return-spring attached to the floor underneath the tank. A lever arm, hung up in the conventional manner, was attached to a loop in the wire underneath the tank, and a celluoid point recorded the movements on a double-drummed kymograph at an increased amplitude of 2.5 times the true one. The kymograph was set at a speed that permitted the recording of the first 4–6 hours of a seastar attack.

Results

More than 80 clams were mounted and offered as food to *Evasterias*, using the first type of recording described above. In many cases, however, the seastars managed to wriggle or tilt the clam out of position, in some cases even straightening out the strong hooks. In other cases they broke the shells, a process facilitated by the drilling and filing. Thus, from the start it was evident that force was used by the seastar. In other cases the adductors were torn at the very beginning of the attack, as observed by Schiemenz (1896) for *Marthasterias glacialis* opening *Venus* sp. Some experiments were stopped to examine the condition of the clams, but 21 curves were secured which show movements of the upper valves during all phases of a seastar attack.

One of the resulting curves is shown in Figure 3. This experiment began at 21:15 when several seastars were placed in the tank, but it was not noted when the attack began. The clam was opened to a gape of less than 1 mm prior to the main opening which began at 23:15, a fact which does not show up on the figure due to the necessary reduction. This very small initial opening which was also noted on two other curves may indicate partial failure of the seastar in its first pulling attempt. Beginning at 23:15, however, it will be seen that the clam was pulled open to a gape of about 10 mm in 12 minutes, the amount of gape being expressed as the distance between the valves at the midventral edge where the nylon line was attached. This latter part of the curve illustrates the typical way in which the opening takes place (cf. Figs. 4 and 7). At about noon the next day the seastar was seen to move away from its now empty prey. The steady but slight drop of the curve thereafter is caused by the ligament slowly pushing the valves further apart. Although the curves differ in some respects they all agree on some important points: (1) once a

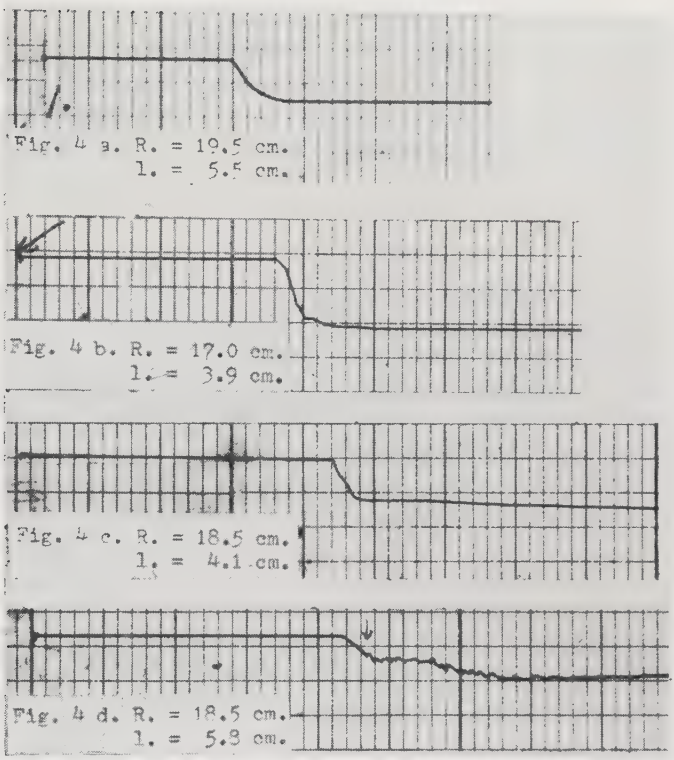

FIG. 4. Kymograph records of movements of bivalve shells during initial period of seastar attacks. Symbols as in Fig. 3.

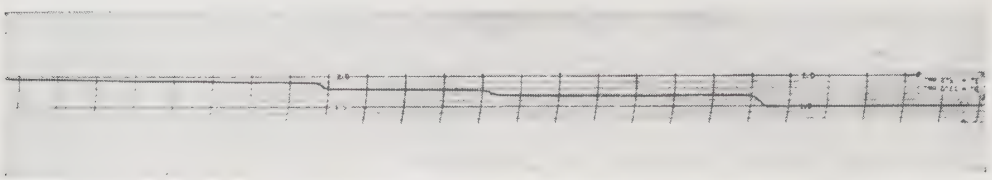

FIG. 5. Kymograph record of movement of bivalve shell during seastar attack. Distance between vertical lines equals 30 minutes of recording. Distance between horizontal lines is 3.0 mm. Other symbols and true gape as in Fig. 3.

clam had been opened it never completely closed again, although partial closing did occur; however, this might not necessarily have been the case if larger clams had been used; (2) in every case the opening took place within a short period of time once the seastar had started pulling. Figure 4b for instance shows that this clam was opened to a gape of about 19 mm in about 8 minutes. The curves do not show any pulling that may take place before the prey actually begins to open, but observations show that this period of time is short, usually lasting but a few minutes with clams of the sizes used in these experiments. However, with smaller seastars or larger clams the period may be expected to increase considerably.

As indicated above, clams are not always opened to a large gape by the first pulling attempt of the seastar. Thus Figure 5 shows a case where the full opening was accomplished in three distinct steps. The important thing to note, however, is the fact that the two secondary openings were accomplished in a short period of time also.

However, to present data on the speed and extent of the initial opening (Table 1), it is necessary to define the latter term arbi-

TABLE 1. *Extent and speed of initial openings of* Protothaca staminea *by* Evasterias troschelii

Experiment no.	*R* of *Evasterias* in cm	Length of shell of *Protothaca* in cm	Time preceding initial opening in min.	Gape of initial opening in mm	Duration of initial opening in min.	Speed of initial opening in mm per min.
1	19.5	5.2	292	3	7	0.4
2	19.5	5.2	37	12	9	1.3
3	19.5	4.9	40	6	6	1.0
4	20.0	5.2	72	8	11	0.7
5	20.5	4.9	140	9	11	0.8
6	13.5	5.4	124	1	5	0.2
7	19.5	5.2	14	11	11	1.0
8	16.0	5.2	57	9	15	0.6
9	22.0	5.4	33	8	3	2.7
10	21.0	4.9	37	4	6	0.8
11	18.5	5.8	87	9	7	1.3
12	17.0	5.2	168	10	12	0.8
13	19.5	4.0	120	9	6	1.5
14	13.0	4.4	128	2	3	0.7
15	18.5	4.7	100	1	3	0.3
16	19.5	4.2	66	1	1	1.0
17	19.5	5.5	65	13	14	1.0
18	18.5	4.1	87	13	9	1.4
19	17.0	3.9	75	19	8	2.4
20	21.0	5.0	?	9	17	0.5
21	18.5	4.5	49	2	5	0.4
Mean values:			89.6	7.6	8.0	0.99

trarily. It is here understood as that part of the curve from the point where the opening begins to the point where the curve again takes a horizontal direction, be it only for a very short distance. It is realized that fixation of the latter point in some cases is a question of judgement but no better procedure seems available. Since very small movements were not recorded on the curves which are the basis for the data in Table 1, the data on the amount of gape of the initial openings are only given to the nearest mm. The time periods given as preceding the initial opening refer to the time elapsed after the seastars were placed in the tank and the kymograph was started. It gives no information about the time elapsed after a seastar had actually come into contact with the clam and the beginning of the opening. However, on numerous occasions during these and other experiments, *Evasterias* has been observed to have its prey fully opened in less than 20 minutes after contacting it. In Expt. 7, Table 1, for instance, the initial opening began within 14 minutes of the start of the experiment.

Table 1 also shows that the mean speed of the openings in 21 experiments was nearly 1 mm per minute, while the fastest opening on record (Expt. 9) was accomplished by an *Evasterias* with an *R* (radius of longest arm) of 22.0 cm which opened a *Protothaka* 5.4 cm in length to a gape of 8.0 mm in 3 minutes, a mean speed of 2.7 mm per minute. The table shows only the amount of initial gape. Where this is small, the seastar usually begins pulling shortly afterwards, sometimes making it difficult to demarcate the initial opening as defined above.

Some typical openings are illustrated in the curves in Figure 4. As seen in Figure 4d, the clam may try to close vigorously during the initial opening and for quite some time thereafter. For the smaller clams, as for instance those in Figures 4b and 4c, no indication of resistance was visible on the curves. Figure 6 shows a very exceptional case, where the clam evidently tried to close almost constantly during the first three hours and then at longer intervals thereafter, the last closing movement occurring 7½ hours after the beginning of the initial opening. During that period the clam evidently succeeded in keeping almost closed, leaving only a small opening for the seastar's stomach to enter.

This resistance of the prey is, however, much better illustrated on the curves obtained by the second method of recording described above. Seven recordings were obtained of which five have been lost; the two remaining are given in Figure 7. The five others were essentially similar with the exception that two of them showed several secondary openings comparable with those shown in Figure 5. In no case was any resistance of the prey recorded at more than four hours after the beginning of the initial opening. The reaction of the clam seems to be essentially similar to the reaction against a constant heavy weight keeping the valves apart. This becomes evident when comparing the two curves in Figure 7 with Figure 6 in Tamura's (1929) paper, which shows the closing movements of a 17 cm oyster against the weight of 10 kg attached close to the margin of one of the valves. In both cases the general extent of the gape is constant for long periods of time and the

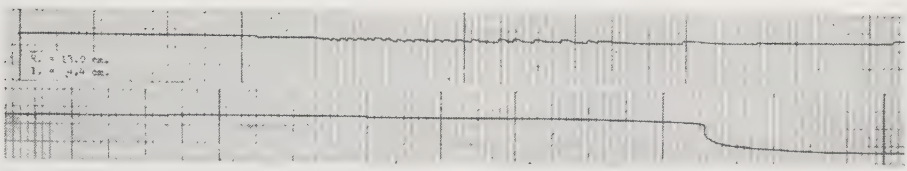

FIG. 6. Kymograph record of movements of bivalve shell during seastar attack. Symbols as in Fig. 3.

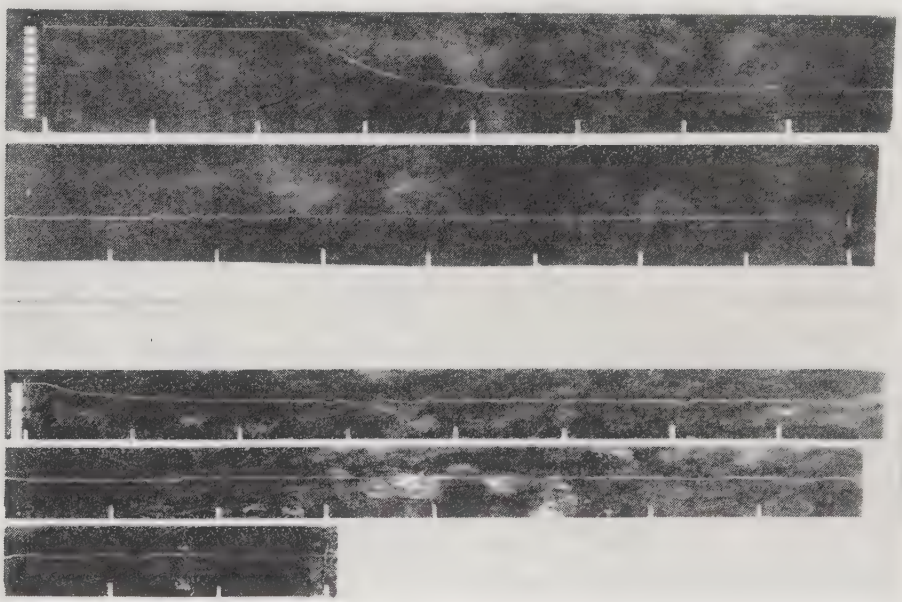

FIG. 7. Kymograph records of movements of bivalve shells during seastar attacks. Distance between marks at bottom equals 10 minutes of recording. Distance between marks on vertical scales is 5.0 mm. True gape of clam is equal to recorded gape divided by 2.5. The clam used in the upper experiment was about 1 cm longer than that in the lower experiment.

amplitudes of the closing movements are very small. Also, in both cases it is seen that after each quick closing attempt, the bivalve slowly opens to the original amount of gape. Thus, the latter fact is probably not due to an immediate counterpull of the seastar, but may be due to an inability of the prey to hold on to the gain of its closing attempt against the forces already exerted upon it. Naturally this balance can only be expected to occur within certain size relationships of seastar and prey. The similarity of Tamura's curve and the curves in Figure 7 also indicates that the adductors are not narcotized during a seastar attack.

The smaller extent of the initial opening as well as the smaller amplitudes of the closing attempts in Figure 7a (above) as compared with Figure 7b (below) is correlated with size of clam. The clam used in the former case was about 1 cm longer, while the same seastar was used in both cases.

II. KYMOGRAPH RECORDING OF MOVEMENTS OF VALVE OF A FALSE LUCITE "CLAM" OPENED BY SEASTAR AGAINST KNOWN RESISTANCE

Methods

These experiments were designed to measure the forces exerted by *Evasterias* in short and prolonged pulls.

A dummy "clam" was constructed of lucite as shown in Figure 8. The hinge consisted of a stainless steel rod, which prevented any tilting of the valves in relation to one another. When closed, the valves fitted tightly together except for a few channels of less than 0.1 mm diameter

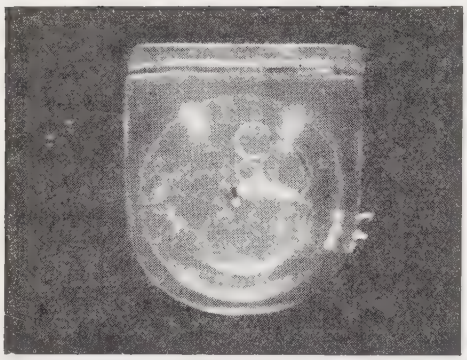

FIG. 8. Lucite dummy "clam" photographed in tank. Note trapped tube feet.

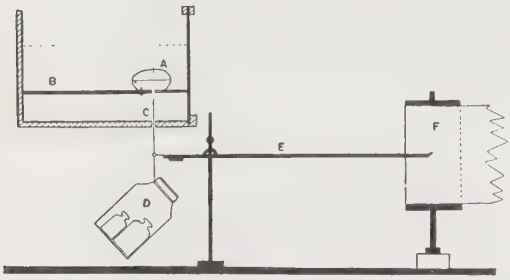

FIG. 9. Experimental setup for recording shell movements of false "clam" during seastar attacks (cf. text).

which were filed to insure that a seastar could sense the *Mytilus* meat inside.

The "clam" (A) in the experimental setup (Fig. 9) was firmly attached to the false bottom (B) by two screws fitting into holes drilled and threaded in the lower valve. A thin, stainless steel wire (C) was attached approximately in the center of the upper valve. From there it ran through holes in the lower valve, the false bottom, and the real bottom of the tank. On the end of the wire was hung a large glass jar (D) of known weight into which different weights could be placed. A lever arm (E) recorded movements of the upper valve on the electric, double-drummed kymograph (F) at an increased amplitude of four times the true one.

The seastars may have had a mechanical advantage in lifting the different weights. However, *Evasterias* did not concentrate its pulling on the ventral area of the upper valve where it would have been most effective. Since the tube feet, as far as could be determined, were distributed pretty evenly around the point of attachment of the wire, the true force exerted in each case probably did not differ significantly from the weight the seastar was lifting.

The lack of a guiding line on the kymograph paper sometimes made it difficult to determine from the resulting curves whether the "clam" had been closed or not at certain points. When using heavy weights, the false bottom tended to sink a few mm during the experiment, adding to the difficulties. However, these difficulties had no influence on the main results as stated below.

As it had been found that *Evasterias* fed more readily at night, all experiments except the last one were started late in the evening. With few exceptions the experiments were stopped before all the *Mytilus* meat in the "clam" had been digested.

Results

The first experiments were done without a kymograph and with weights not exceeding 500 grams to determine whether seastars would open such a false "clam" at all. Both *Pisaster ochraceus* and *Evasterias troschelii* opened it, for the "clam" was emptied of its content of *Mytilus* meat and tube feet were found trapped between the valves as seen on Figure 8.

Using *Evasterias* only, the remaining 14 experiments were recorded on the kymograph, the weights gradually being increased from 700 to 5500 grams.

The curve in Figure 10 shows the typical opening movements obtained and was chosen for illustration because it shows more activity of the seastar than in any of the other experiments of this kind. The seastar used had an R of 22 cm, and the weight attached to the wire was 1800 g. While the live clams of the sizes used in the experiments referred to in Section I never succeeded in closing their valves once they had been opened, Figure 10 shows that the false "clam" closed several times, and this was common in all the experiments of this section. The obvious explanation for this is: (1) that the weight in most cases probably exceeded the resistance that a live clam of comparable size could exert, and (2) that the

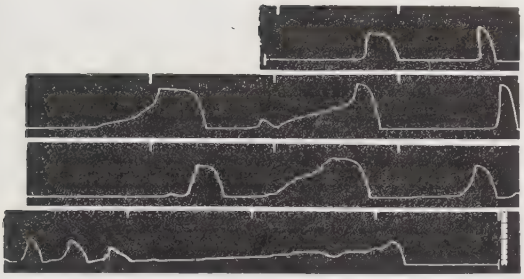

FIG. 10. Kymograph record of movements of freemoving valve of false "clam" during seastar attack. Distance between marks at bottom equals 60 minutes. Distance between marks on vertical scale is 5.0 mm. True lift is equal to recorded lift divided by 4.

seastar with a live clam pulls against a gradually diminishing force, whereas it pulls against a constant force to open and keep open the false "clam." This tiring of the seastar is probably also responsible for the fact that the intervals in which the "clam" was held open became shorter towards the end in most of the experiments.

There is, however, one striking similarity between the curves derived from experiments with live clams and those from the false "clam." The parts of the curves illustrating the openings are practically identical. In both cases the openings take place within a short period of time and in both cases the speed of the opening is fastest during the beginning of the pull, gradually becoming slower towards the end of it. This indicates that the slowing up in opening a live clam is not due to increased resistance of the adductors but is due to the method used by the seastar in pulling the valves apart.

Table 2 gives some data obtained by studying the resulting curves from all 14 experiments. While the time elements involved could be read directly, the true amount of gape, or rather, distance of lift, was arrived at by dividing the measured vertical distances on the curves by 4 to correct for the 4x amplification by the writing lever.

The first column under the heading "Measured data" shows the maximum distance the different weights were lifted in each experiment, while the next two columns shows the extent and duration of the fastest lift in each case. Finally the last column shows the speed of the fastest lift as expressed in mean number of mm the weight was lifted per minute during the total time period of the lift concerned.

It will be seen that the weights were maximally lifted anywhere from 2.8 mm to 13.5 mm and that the mean of the maximum lifts for all experiments was 6.6 mm.

The *extent* of the lifts did not decrease with increase in weights applied to the upper valve. Nor did the *speed* of the lifts as measured in mm per minute show any decrease with weights up to 4500 g. However, it was somewhat slower with weights of 5000 and 5500 g, which may indicate that weights of the latter magnitude are close to the limit that an average sized *Evasterias* can lift. Seastars were seen straddling the "clam" for two nights before the 5000 grams was finally lifted on the third day. However, these few data and observations do not warrant definite conclusions on the maximum pull an *Evasterias* of average size can exert.

TABLE 2. *Extent and speed of lifts of different weights lifted by* Evasterias *while attacking a false "clam"*

Weight lifted in g	*R* of *Evasterias* in cm	Measured data				Converted data (cf. text)			
		Maximum lift in mm	Extent of fastest lift in mm	Duration of fastest lift in min.	Speed of fastest lift in mm per min.	Maximum lift in mm	Extent of fastest lift in mm	Duration of fastest lift in min.	Speed of fastest lift in mm per min.
700	23.5	8.8	8.8	6.2	1.4	13.5	13.5	6.2	2.2
900	21.0	5.6	5.6	7.3	0.8	8.6	8.6	7.3	1.2
1000	21.0	6.3	4.8	4.2	1.1	9.7	7.4	4.2	1.8
1200	21.5	7.8	7.8	6.2	1.3	12.0	12.0	6.2	1.9
1400	22.0	2.8	2.8	13.5	0.2	4.3	4.3	13.5	0.3
1600	22.0	6.2	5.0	4.2	1.2	9.5	7.7	4.2	1.8
1800	22.0	10.6	10.6	8.8	1.2	16.3	16.3	8.8	1.9
2000	17.5	8.5	8.5	12.0	0.7	13.1	13.1	12.0	1.1
2500	17.5	4.9	4.9	11.4	0.4	7.5	7.5	11.4	0.7
3000	17.5	3.5	2.9	7.8	0.4	5.4	4.5	7.8	0.6
3500	22.5	13.5	13.5	17.2	0.8	20.8	20.8	17.2	1.2
4500	22.5	7.0	6.4	6.2	1.0	10.8	9.8	6.2	1.6
5000	22.5	3.9	3.9	23.4	0.2	6.0	6.0	23.4	0.3
5500	22.5	3.3	3.3	26.6	0.1	5.1	5.1	26.6	0.2
Mean values		6.6	6.3		0.8	10.2	9.7		1.2

A comparison of the data in Columns 3 and 4 of Table 2 reveals the interesting fact that the fastest lift most often also was the maximum lift recorded in the particular experiment.

As the wire was attached to the approximate center of the "clam" the data referred to above pertain to this point, *i.e.* the lifts recorded are not identical to the gape of the "clam" at the midventral edge. However, it is of considerable interest to compare the results of these experiments with those recorded for the experiments with live clams. In a *Protothaca* about 5 cm long, which is within the size range used in the latter experiments, the distance from the hinge to the midventral edge, where the nylon line was attached, is about 4 cm, as against the distance of only 2.6 cm from hinge to the point where the wire was attached in the false "clam." Consequently the data on extent of gape of the false "clam" may be made comparable to those from a live clam of the mentioned size by multiplying the measured amount of lift by 1.54. These data are given in Table 2 under the heading "Converted data." As the absolute time involved in opening the "clam" is not affected, the data in Column 3 are identical with the corresponding column of the measured data. A comparison with the results given in Table 1 shows that the speed of the opening is just as fast or faster for the false "clam" as for the live clams, at least with weights up to 4500 g. In both cases the mean speed of the openings seems to be about one mm per minute as measured at the midventral edge.

The experimental method employed here makes it possible for the first time to measure exactly how much a seastar of a certain size can pull, not only momentarily, but also in time. It provides measurements of the total period of pulling and holding against a given force over a longer period of time, and since weight, distance of lift, and the time involved can be accurately measured, the amount of work done can be expressed in normal physical terms. Some data of this type are given in Table 3.

It will be seen that there was no decrease in the ability of the seastar to hold open the "clam" with increase in weights up to the 5500 g used. Nor was there any decrease in the total time a seastar could keep the "clam" open as determined in per cent of the duration of the experiment. However, the last two experiments were of shorter duration than the preceding ones. It is certainly noteworthy that an *Evasterias* can keep a weight of 4500 g lifted for 5 hours and 43 minutes, and that this same weight was lifted within 14.5 minutes of the start of the experiment, a period of time which includes contacting the "prey" and making necessary preparations for the lift.

Since a seastar inserts its stomach as soon as a prey is open and withdraws it again as soon as the soft parts are digested, the method employed in these experiments could also be used to determine the rate of digestion for species of seastars with extra-oral digestion. This would involve placing known amounts of food in the "clam" and determining the duration of stomach eversion, which should correspond roughly to the

TABLE 3. *Duration of closed and open stages of the false "clam" during experiments with* Evasterias *lifting different weights (cf. text)*

Weight lifted in g	*R* of *Evasterias* in cm	Duration of experiment in min.	Time preceding initial opening in min.	Maximum period of constant lift in min.	Total time of lifts in min.	Total time of lifts in per cent of duration of experiment
700	23.5	825	35.5	599.0	633.0	76.7
900	21.0	675	154.0	107.0	427.0	64.7
1000	21.0	1275	109.5	258.0	953.5	74.8
1200	21.5	780	73.0	651.5	651.5	83.5
1400	22.0	685	52.0	633.0[1]		
1600	22.0	906	40.5	615.0	623.5	68.8
1800	22.0	845	46.0	206.0	471.5	55.8
2000	17.5	830	41.5	180.0	484.5	58.4
2500	17.5	840	17.0	385.0	570.5	67.9
3000	17.5	820	56.0	239.0	679.0	82.8
3500	22.5	930	46.0	284.0	482.0	51.8
4500	22.5	980	14.5	343.5	615.0	62.8
5000	22.5	485	88.5	166.5	346.5	71.4
5500	22.5	140	0.0[2]	91.0	129.5	92.5
Mean values		786	59.3	340.0	543.5	70.1

[1] Fragments of a byssus gland prevented the "clam" from being closed during part of the experiment, the seastar taking advantage of it.

[2] The last experiment was done in continuation of the preceding one, 500 g being added while the seastar was already lifting 5000 g.

time the "clam" was found to have been open. The use of light weights would probably be necessary to insure maximum opening of the "clam" by the seastar.

III. OBSERVATIONS OF SEASTARS FEEDING THROUGH A SCREEN

Methods

These experiments were designed to study the behavior of *Evasterias* when force could not be utilized in opening bivalves.

A strong cotton net with 8 mm mesh was strung over a rectangular, wooden framework as long as the tank. *Mytilus edulis* 4 to 6 cm long were allowed to attach firmly to one side of the net, which was then placed in the tank with *Evasterias* on the other side of it. The frame was placed close to the front of the tank to facilitate observation of the seastars. Experiments were also conducted with *Protothaca* 4 to 5.5 cm long, which were placed on crossbars of the frame as shown in Figure 11.

Although the seastars could pull on their prey, in rare cases making an inward dent in the net of as much as 2 cm, they could not possibly pull on both valves in opposite directions at the same time. Over a period of 3 months, more than a hundred bivalves were offered to the seastars.

Results

Fasmer's (1950) and Feder's (1955) observations that *Pisaster ochraceus* may feed on bivalves without opening them by force was also found to be valid for *Evasterias*.

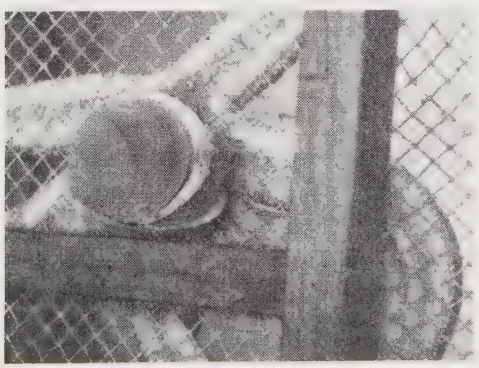

FIG. 11. *Evasterias* feeding on a clam through a screen (cf. text).

As stated earlier, *Evasterias* usually opens and starts digesting a prey of the size mentioned above within 20 minutes after contact. In these net experiments, however, it often took several hours before the seastars inserted their stomach into the prey. When they were feeding on clams, this time period often extended to 12 hours or more. In other cases the seastars left their prey unharmed after they had been clinging to them through the net for many hours. This was the case especially when clams were placed on the crossbar with their hinges against the net facing the seastars. In other cases the seastars would manipulate the prey with their tube feet until they had turned it into a more favorable position with the hinge facing more or less away from the net.

However, when given mussels as food, the seastars often fed more rapidly because they utilized the large permanent opening of the byssus region as an entrance for their stomach. If any permanent opening is present in *Protothaca* it must be very small in comparison. Even in *Mytilus*, the permanent opening was not always utilized, as the stomach was often inserted between the valves at the posterior end, and in such cases several hours usually passed before this was accomplished.

The digestion period was also considerably lengthened. Thus, seastars with *R*'s varying from 17 to 24 cm required 15 to 22 hours to digest a clam as compared with the normal period of 7 to 15 hours.

IV. FEEDING BY SEASTAR WITH STOMACH CONTACT ONLY

Method

This experiment was designed to see if *Evasterias* can feed on a bivalve by use of its stomach only.

A *Mytilus edulis* 5.4 cm long was placed inside a plasticine tube about 8 cm long. The tube was open at one end, leaving a distance of about 2 cm from the entrance to the anterior end of the enclosed mussel. It was closely fitted to the mussel except on its ventral side where a space of about 1 cm in width was available for the eventual passage of a seastar stomach. The tube was then placed in a tank with *Evasterias*.

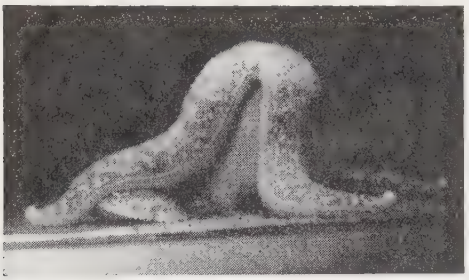

FIG. 12. *Evasterias* feeding on a *Mytilus* enclosed in a plasticine tube (cf. text).

Results

As seen in Figure 12 one of the seastars raised the tube on end with the opening towards the mouth, inserted its stomach through the hole, down along the ventral side of the mussel and through the byssus opening. In this manner the mussel was totally digested. Two similar experiments yielded the same results.

Thus *Evasterias* can feed on molluscs with permanent openings as long as it is able to reach them with the stomach. As *Evasterias* is especially common in the littoral zone of rocky coasts where its prey is often found in crevices, this factor may be of importance in nature. It is able to evert its stomach to a distance equal to at least half the length of its arms. Mead (1899) writes that *Asterias forbesi* can evert its stomach to the full length of its arms, and Schiemenz's (1896) Figure 5 indicates that the same is true for *Marthasterias glacialis*.

ON THE METHOD EMPLOYED BY *EVASTERIAS* IN OPENING BIVALVES BY FORCE

The method of opening attached clams was observed early in these studies. Figure 13 shows the most important stages involved.

First the seastar places itself with its mouth opening over the upper, free-moving valve. A mass of tube feet is attached to the latter whereupon the seastar starts humping up. Since the tube feet are firmly attached, this results in their stretching, usually to a length far exceeding that attained by compressing the ampullae. They may be stretched mechanically to about twice their normal protracted length without rupture of the column wall in *Asterias rubens* (Smith 1947) and this would seem to be the case with *Evasterias*, too. Figure 13b shows the position of the seastar in relation to the clam at the end of this stretching stage. No force has as yet been brought to bear on the prey, except for the pull required to stretch the tube feet. The actual pull is now accomplished by retraction of the attached tube feet, the ultimate result being the opening of the clam as shown in Figure 13c. It is thus the longitudinal musculature of the tube feet which exerts the force.

The method of opening unattached bi-

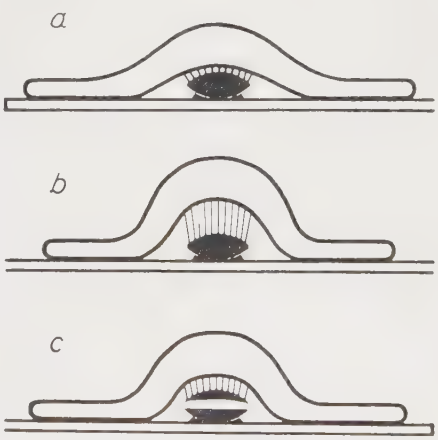

FIG. 13. Schematic drawings of stages of the opening of an attached clam by *Evasterias* (cf. text).

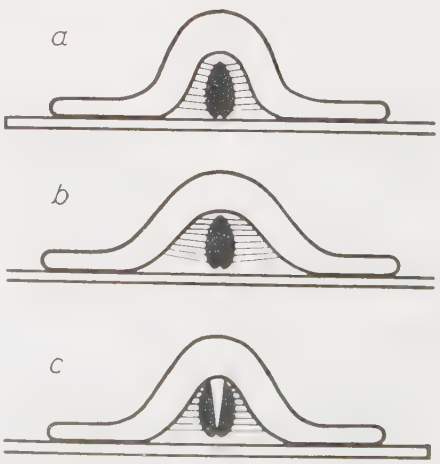

FIG. 14. Schematic drawings of stages of the opening of an unattached clam by *Evasterias*.

valves is essentially the same and is shown in Figure 14. First the hinge is invariably turned away from the mouth opening, as observed for *Asterias* by Hamann (1885). Then a mass of tube feet is attached to each valve as shown in Figure 14a. Next the seastar humps further up and moves the inner parts of the arms away from the clam while keeping the tube feet firmly attached to it. A stretching of the tube feet therefore results, and the position of the seastar in relation to the clam reaches the stage shown in Figure 14b. Then the attached tube feet are retracted and the clam is opened as shown in Figure 14c.

The retraction of the tube feet results of course in a pull on the seastar itself. If it were unable to resist this pull, it would simply pull itself into close contact with the prey without accomplishing the opening. This obviously did not happen with the clams used in the present studies, although a partial sinking of the disc was often observed when a seastar opened the false "clam," as illustrated in Figure 13. In the case of seastars opening unattached clams, a compression of the inner parts of the arms towards each other during the pull was observed clearly in a few cases. It is indicated in Figure 14 to emphasize that only the musculature of the tube feet is responsible for the pull, and not a straightening of the arms as postulated by Mead (1899). Nevertheless, *Evasterias* can lift and hold considerable weights for several hours without collapsing (cf. Table 3). How is it capable of resisting such forces?

Observations in the laboratory leave no doubt that the seastar attaches very firmly to the substratum with the tube feet of the distal parts of the arms whenever it is opening a bivalve, and this factor may be of some importance. On a loose bottom this would be impossible. It is much more likely that an internal mechanism is responsible for the necessary rigidity of the seastar when pulling. Thus Schiemenz (1896) writes about the humped-up feeding position of *Marthasterias glacialis*, "dass der Berg ausserordentlich starr ist und einen Versuch ihn einzudrücken ungeheuren Wiederstand leistet." Hancock (1955) states that *Asterias rubens* taken from the dredges were frequently found in the characteristic feeding position although the prey had usually been released. That such a stiffness exists and will persist for some time after the seastar has released its prey, strongly indicates that these seastars are able to "lock" themselves before starting the pull. Whether such a locking can be accomplished by the musculature alone or in combination with a possible interlocking mechanism of ossicles in the endoskeleton remains to be seen.

Although Figure 14 illustrates the underlying principles of a forceful opening, many variations in the position of the seastar while pulling may be observed. Thus on small prey the pull may be exerted in opposite directions by the tube feet of only two of the arms, and the mouth opening may be somewhat off to the side as observed from above. In fact, Feder (personal communication) has seen two-armed *Pisaster*'s open small *Mytilus* by force. Little is left of the humped up feeding position in such cases.

The force which *Evasterias* can exert, as determined by the experiments with the false "clam" is more than sufficient to open bivalves within the size range it normally feeds on. Schiemenz (1896) showed that specimens of *Venus* sp. up to a length of 4.0 cm would open up a few mm or more within 10 minutes if subjected to a pull of only 900 g at the ventral margin of the shell. Reese's (1942) criticism on this point cannot be considered valid, because he disregarded openings up to as much as 7.0 mm, stating that it would be quite impossible for a seastar to insert its stomach through such small openings. Feder (1955) who tested the strength of *Mytilus* states that a pull of 4000 g can generally open mussels of up to 10 cm length, but that several hours of pull may be needed to overcome the adductors of those in the 9 to 10 cm range. Observations of *Evasterias* feeding on *Mytilus californianus* of 12 cm or more in length, indicated that the seastars could not open these by force at all. They fed through the byssus openings and no gape of the mussels was observed in any case before at least 5 hours,

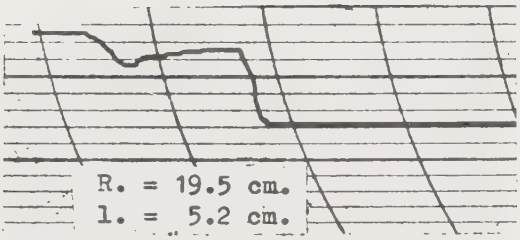

FIG. 15. Kymograph record of the initial opening of a clam by *Evasterias*, resulting in the tearing of the adductor muscles (cf. text). Symbols and true gape as in Fig. 5.

and often a considerably longer period had passed after the initial insertion of the seastar stomach. Feder's (1955) observation that a 4000 g pull exerted at the byssus region would not open a 13.1 cm *Mytilus* in 8 hours indicates the same thing. On the other hand, with the pull exerted at the posterior region, where *Pisaster*, according to Feder, often concentrates its pull on large mussels, a *Mytilus* of 13.0 cm opened about 0.2 mm in 15 minutes. In evaluating these data it should be pointed out that the strengths of the adductors in *Mytilus* are probably considerably greater than in clams (Tamura 1929).

As also observed by Schiemenz (1896), the adductors are often torn when the prey is opened. Such a case with an *Evasterias* opening an attached *Protothaca* is recorded in Figure 15. After an initial opening to an 11.0 mm gape, the clam apparently succeeded in a partial closing. The seastar then more completely retracted the stretched tube feet, and in less than 8 minutes the clam gaped an additional 19 mm. Both adductors were torn during this period, the process taking place so quickly that the movement of the upper free-moving valve could easily be observed during most phases of the pull. While one adductor snapped early the other was not completely torn before the very last minute of the pull. In another case a seastar with an R of 20 cm exerted a pull on a 4.9 cm mounted clam, which within 6 minutes resulted in the breakage of both the hinge and the ligament as well as the tearing of one of the adductors.

It seems evident that the power that can be exerted by any one seastar is dependent on the pulling power of each individual tube foot and the number of them that are attached in an effective pulling position. Preyer (1886) estimated that the maximum pull of a single tube foot of *Asterias* (size unknown) was 25 g. Paine (1926) estimated that the mean adhesive power of a tube foot of the same genus was 29.4 g, or 17.7 g per square mm of sucking disc. These data, which need corroboration, indicate that only about 200 tube feet would be necessary to lift and hold a weight of 5000 g. However, since the tube feet may work in relays, as first suggested by McBride (1906), and since all the tube feet attached to the prey cannot be brought into the same effective pulling position, the actual number would probably be much higher.

Although more tube feet may find a place on larger prey, this extra pulling potential is counteracted by two factors, viz. the increasing power of the adductors and the difficulty in attaching the tube feet in an effective stretched pulling position. If the prey is above a certain size in relation to the seastar, a proper pulling position cannot be assumed, as noted by Schiemenz (1896).

OTHER OBSERVATIONS AND A GENERAL DISCUSSION OF THE EXTRA ORAL METHOD OF FEEDING

Many points concerning the digestion in seastars are still unknown, but that it is mainly extracellular is evident (Anderson 1953). The mechanism whereby the stomach is everted and retracted has also been studied and reviewed by Anderson (1954) and is now fairly well understood.

However, widely different opinions have been held as to the time of eversion of the stomach in relation to the stage of attack. De Montauge (1879) postulated that it was everted immediately and wrapped around the prey to suffocate it. Others have held that it was everted at the very beginning of the attack to facilitate the secretion of a toxic substance. Recently Aldrich (1954) stated that the stomach is everted even before the tube feet are properly attached to the prey. Schiemenz (1896) on the other hand emphasizes that the stomach is not everted before the prey is actually gaping,

and he has been supported by McBride (1906), Mortensen (1927), Galtsoff and Loosanoff (1939), and Hancock (1955) among others.

These apparently contradictory statements fall readily into line in view of our new knowledge. When using force, *Evasterias* never everted the stomach before the prey was actually gaping, but, when it fed on large mussels through the byssus opening, the stomach was everted soon after the seastar contacted the prey, and long before the prey was observed to gape. The only exceptions were those cases where an opening by force was evidently attempted first.

Prior to the attacks on clams as well as on the false "clam" used in these studies, and during the stage of the attack when the tube feet are being stretched by the humping up of the seastar, it was often observed that some of the unattached tube feet were engaged in feeling along the ventral margins of the clam. This indicates that receptor cells, which are especially abundant in the tips of the tube feet (Smith 1947), register the beginning of the opening and transmit stimuli to induce the eversion of the stomach. The finding of a permanent opening by such tube feet could stimulate the seastar to evert its stomach without an opening of the prey. Usually tube feet move into the cavities of the prey as soon as the latter begins to gape and simultaneously with the eversion of the stomach. As described by Smith (1945, 1950) the tube feet are capable of independent activities to an extraordinary degree.

Statements that the stomach of a seastar has been caught between the valves of a bivalve are not uncommon in the literature. However, even with the heaviest weights used in the experiments with the false "clam," not a single case of this kind occurred, although tube feet were caught (cf. Fig. 8). If a seastar is able to open a certain prey by force it is also able to protract its tube feet slowly enough to permit the withdrawal of the stomach, although the protraction is a faster process than the retraction as is illustrated by the curves in in Figure 10. Observations of trapped stomachs of seastars taken out of aquaria for observation cannot be accepted as valid as such treatment may result in a sudden release of the seastar pull (Hancock 1955). Furthermore, Feder (personal comm.) points out that if a stomach is inserted through a very small permanent opening, an observer might consider that the stomach is trapped.

Since seastars have extracellular digestion it is important that as much of the stomach as possible contacts the tissues of the prey. In seastars with intraoral digestion this is accomplished by enveloping the prey in pockets of the stomach wall (Christensen 1954), while in seastars with extraoral digestion each of the five stomach lobes is inserted as deeply into different cavities of the prey as possible. As *Evasterias* is unable to insert all lobes through openings below a certain size, digestion slows down under such circumstances. This was clearly indicated in the experiments with *Evasterias* feeding on clams through a net, where the digestion period was considerably lengthened. It is also seen by comparing the cases illustrated in Figure 15 and Figure 6. In the former a highly-gaping clam was digested in about 7 hours, while in the latter, where only a very small opening was available, the digestion lasted for at least 16 hours although this clam was smaller than the other.

These considerations indicate why *Evasterias* applies force in attacking clams and mussels whenever circumstances permit it even though a permanent opening may be available. It appears that force is the primary method employed by seastars of the *Asterias* type to gain access to the tissues of their bivalve prey, as it seems the only method by which they can attack and digest such prey in a minimum period of time.

Another factor remains to be considered. Feder (1955) states, "Since the stimulus of a starfish as well as a strong mechanical pull does not appear to overcome the natural tendency of the bivalves studied to gape, it appears that voluntary opening on the part of these molluscs must play an important role in the feeding process of the starfish." He found (personal comm.) that Pismo clams very often remain open while *Pisaster* picks them up, carries them around, and

even begins to digest them. No observations of this kind were made in these studies, but the question certainly merits attention.

Feder (personal comm.) also permits me to quote another important observation. He repeatedly found that stimulation of the mantle would not cause a clam to close immediately when a *Pisaster* was in the act of pulling the valves apart. As it has been shown by Schiemenz (1896), Reese (1942), and Galtsoff (1952) among others that a number of bivalves, including clams and oysters, may close immediately when lightly touched on the mantle even several hours after the beginning of a prolonged pull on the valves by weights or seastars, this indicates that a distinction should be made between the pulling and the holding phases of a seastar attack. This also merits attention.

Of more than 50 specimens of *Evasterias* used in these studies, comparatively few would feed under all circumstances. In spite of excellent aquarium conditions, a few would not feed at all for several months, and some would not feed on the false "clam." Some possibly reduced their feeding during ripening of their sexual products as known for *Asterias forbesi* (Galtsoff and Loosanoff 1939). Mortensen (1921) found that *Evasterias* had ripe sexual products by the end of May at Nanaimo, and a male spawned in a tank at Friday Harbor on May 22, 1955. Thus gonad development was apparently occurring during the studies reported here. These observations emphasize the need of a large number of experimental animals for such studies.

The main results of Feder's (1955) study on *Pisaster* and the results of these studies on *Evasterias* will probably prove to be valid for all those species of seastars of the family Asteriidae that possess four rows of tube feet. The latter is probably an evolutionary adaptation to enable these seastars to open their prey by force more effectively than could be done with only two rows. In fact no seastar with only two rows of tube feet has ever been reported to open its prey by force. Most of the species of Asteriidae are found in the littoral zone where force is not only of value in opening mussels and other bivalves but is also extensively used in pulling barnacles, chitons, and other epifaunal prey off their substratum.

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Biologic and Hydrographic Adjustment in a Disturbed Gulf Coast Estuary[1]

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ABSTRACT

East Bay, an arm of the Galveston Bay system of Texas, was studied during the summers of 1954, 1955, and 1956. The first study was made with the Bay in its natural state, the second investigation followed the excavation of a pass (Rollover Pass) that permitted considerable interchange between the Gulf of Mexico and East Bay, and the third study was subsequent to partial blocking of the Pass.

Considerable evidence from salinity distribution and fluctuations in population density of the dominant fish and shrimp species suggests a rapid physical and biological reversion toward the natural condition, following the blocking of the Pass. In addition, the presence or absence of certain fish and shrimp forms was correlated with changes in the Bay.

INTRODUCTION

During the summers of 1954, 1955, and 1956, studies were conducted of East Bay, a northeastwardly directed arm of the Galveston Bay system of Texas. The investigation, a project of the Marine Division, Texas Game and Fish Commission, was designed to ascertain the effects of the introduction of Gulf waters of high salinity into estuarine East Bay, following excavation of a pass through narrow Bolivar Peninsula (Fig. 1).

The first study was accomplished during June and early July, 1954, prior to the construction of Rollover Pass, and at a time when the Bay demonstrated undisturbed estuarine conditions (Reid 1955a, 1955b).

The Pass was opened in January, 1955, and the second study was conducted during June, 1955, when much of East Bay was under the influence of Gulf of Mexico waters flowing with the tides through Rollover Pass. The biology and hydrography of the Bay was considerably modified from the 1954 condition (Reid 1956). When originally opened in early 1955, the Pass was 1800 feet long, 80 feet wide, and approximately 8 feet deep, with sides sloping 2:1. By the time of our field study in June, 1955, the Gulf entrance of the Pass had been eroded to a width of nearly 300 feet and the channel scoured to depths of 12 or more feet.

Measures taken to control erosion in the vicinity of the Pass dampened the effects of the Pass on East Bay. This was the condition at the time of the June, 1956, East Bay investigation. In the fall of 1955 a series of vertically-sunk, interlocking steel pilings, three feet wide, were placed parallel to, and on the Gulf side of, the highway bridge over the Pass. These pilings were so arranged as to form an almost solid barrier extending from either shore toward the middle of the channel. In the near-center of the barrier, 12 spaces three feet wide permitted flow of water. The depth at this point was six to ten feet (depending upon the stage of the tide). There was then an area of some 200 to 400 square feet between pilings for tidal flow. This restriction of the flow naturally resulted in the building up of a considerable head of water on the up-current side of the barrier, and something of a "jet-effect" or high initial velocity was imparted to the water passing between the pilings. Several determinations indicated a velocity of nearly 75 feet per minute on the Gulfward side, while only a negligible current was detected on the Bayward side as the Gulf tide was ebbing. Current on either side was relatively weak during flood stage of the Gulf tide.

Although the shores of the Pass on the Bayward side of the bridge had remained

[1] A contribution of the Marine Laboratory, Texas Game and Fish Commission, Rockport, Texas.

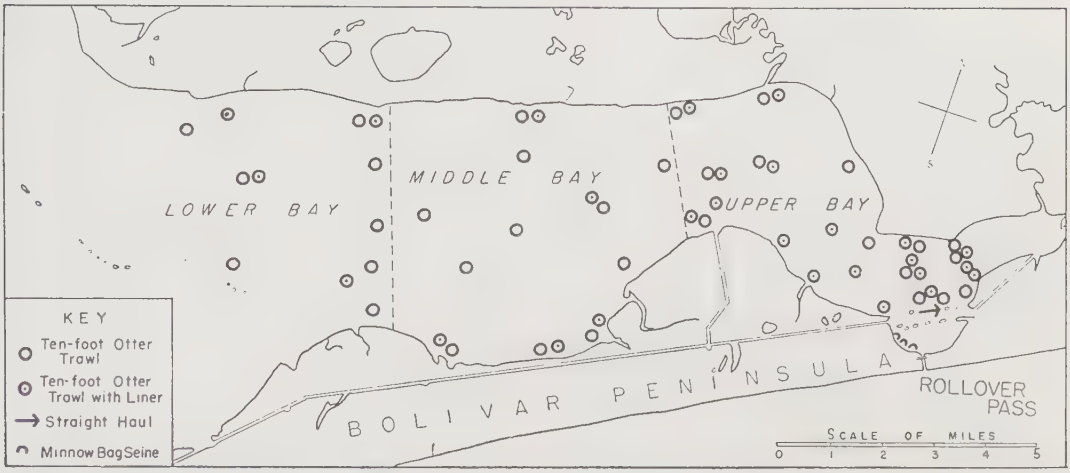

FIG. 1. Map of East Bay, Texas. Collecting stations, gear employed, and location of Rollover Pass are shown.

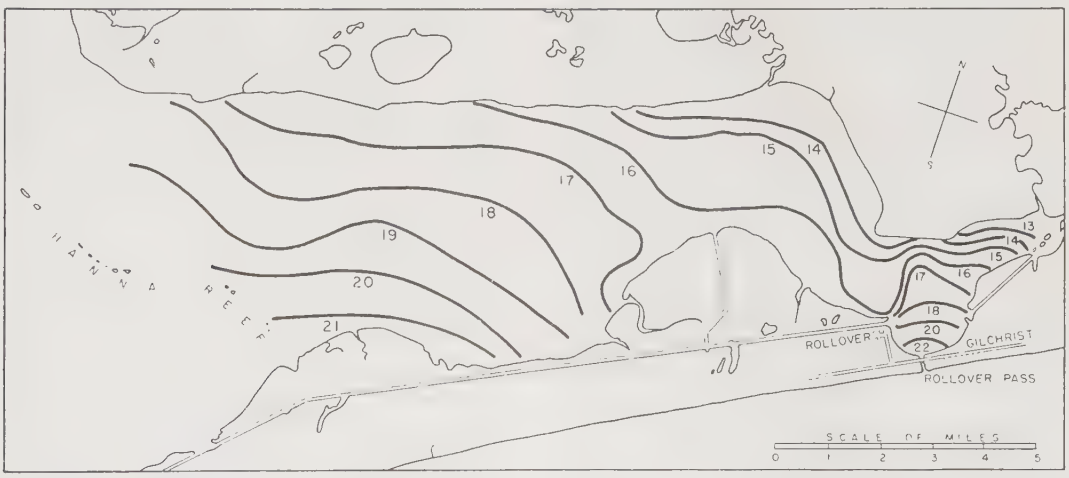

FIG. 2. Salinity distribution pattern in East Bay in June, 1956. Isohalines are based on averages at stations occupied.

relatively stable, tides and currents had considerably altered the physiography of the Gulfward side. During June, 1956, the channel on the Gulfward side was restricted to 15–20 feet near the bridge and a relatively shallow depth. A sand bar developed on the Gulfward side threatened to block the channel completely within a short period of time.

The general extent to which the Pass contributed to the total salinity of East Bay is demonstrated in Figure 2. Comparison of the isohaline pattern derived in June, 1956, with that for the same month in 1955 (Reid 1956), indicates considerable restriction of the incursion of water of high salinity through the Pass during the intervening year. Similarly, the 1956 salinity pattern for most of the Bay indicates an increased influence of Galveston Bay on the salinity of East Bay.

Rollover Pass was faunistically rich. Fishing pressure was considerable, with dozens of fishermen present daily. When the water was "clear," speckled trout (squeteague) were frequently caught, especially underneath the highway bridge. Sand

trout, croaker, spadefish, drum, redfish, pigfish, silver perch, pinfish, big-eyed herring (ladyfish), catfish, shark, ray, eel, and small pompano were taken regularly. Blue crab were commonly caught, particularly along the Bayward sides of the Pass.

Thus, opportunity was afforded to study East Bay (1) in an undisturbed state (1954), (2) when there was great exchange between the Bay and Gulf of Mexico through the Pass (1955), and (3) when the exchange had been considerably diminished by partial blocking of the Pass (1956). This paper presents biologic and hydrographic data pertaining to East Bay during the summer of 1956, and relates these data to previous findings in demonstrating an apparent general readjustment of the Bay toward its original condition.

The author, assisted by Hinton D. Hoese and Roy W. Spears, students at Texas A. and M. College and employed by the Marine Division of the Texas Game and Fish Commission, conducted the field phase of the 1956 investigation with headquarters at Gilchrist, Texas. Analyses of data were made at the Marine Laboratory, Rockport, Texas.

The cooperation of Mr. Cecil Reid, Director; Miss Patricia Pew, Biologist; Mr. Rudy Marek, Chemist; and Miss Mary Frances Bell, Typist, Marine Division, Texas Game and Fish Commission, is acknowledged with full gratitude. Messrs. Thomas Spell and Charles Faggard of Gilchrist, and Game Warden G. B. Killibrew of High Island, gave invaluable assistance during the field work.

Appreciation is accorded Dr. Harold H. Haskin and Dr. Paul G. Pearson, Rutgers University, for criticism of the manuscript.

METHODS

The gear used during the 1956 field study was essentially that employed during the previous studies and described more fully by Reid (1955a, 1956).

In general, a 10-foot otter trawl of standard design and seines were used for collecting specimens. Sixty-one trawl collections were made: 34 hauls utilized the regular codend of one-inch stretched mesh, while 27 collections were made with an inner liner of three-eighths inch mesh hung inside the trawl (Reid 1955a). Each trawl collection was based on a 10-minute tow in a circular path. The seine used regularly was a 40-foot minnow bag seine.

Fish specimens were measured in millimeters from the tip of the snout to the structural base of the caudal fin (Standard Length). Shrimp were measured from the anterior tip of the rostral process to the posterior edge of the telson. Crabs were measured transversely between the tips of the carapace.

Stations occupied and the gear employed during the 1956 investigation are shown in Figure 1. These areas are the same as those studied in 1954 and 1955.

Salinity determinations by the Mohr silver nitrate titration method were made from surface samples collected directly in citrate bottles. A total of 155 samples was taken from the Bay and Intracoastal Waterway. Temperatures were read from a laboratory thermometer held in the water, 171 readings being taken.

EAST BAY HYDROGRAPHY

East Bay, a part of the Galveston Bay system, is approximately 20 miles long from its narrowed head near Gilchrist, Texas, to the junction with Galveston Bay proper. At its lower end East Bay is nearly five miles wide. The Bay lies essentially parallel to the Gulf coast, being separated from the Gulf by Bolivar Peninsula which averages about two miles wide along its 23-mile length. East Bay is shallow, maximum depth at mean low water being nearly seven feet. The bottom is mostly deep mud, sloping gently from the adjacent salt marsh. The physiography and geography of the area have been presented in more detail in earlier reports (Reid 1955a, 1956).

Salinity

Salinity data obtained during June, 1956, pointed to the lesser role of Rollover Pass in the salinity distribution within East Bay. Figure 2 clearly demonstrates that most of the Bay was under the influence of the Galveston Bay system and that only the uppermost reaches of the Bay were affected by

Gulf waters reaching those areas through the Pass (*cf.* Reid 1955a, 1956). Such a condition was expected in view of the partial blocking of the Pass.

The general pattern of the isohalines and the over-all salinity of the Bay during the 1956 study more closely approximated the 1954 condition than that of 1955. Excluding the extreme upper area, the 1954 salinity over most of the Bay ranged from about 10‰ in the upper reaches to approximately 20‰ in the lower Bay. Similarly, the 1956 range for essentially the same area and distance varied from 14‰ to 21‰. During the 1955 investigation the salinity in the upper area averaged near 18‰, grading to approximately 15‰ in the lower region.

It has been pointed out (Reid 1956) that the salinity distribution in the Bay in 1955 was completely reversed with respect to that during the 1954 investigation; the 1956 results were essentially as in 1954. Except for the small Bay area opposite the Pass the salinity gradient, which in 1955 decreased from the upper Bay to the lower, was opposite in 1956.

The general pattern of the isohalines over most of the Bay in 1956 (Fig. 2) agreed with that of 1954. The patterns for both years agree closely with that described by Pritchard (1952) for estuaries of the Chesapeake Bay area, wherein lower salinites are found on the right shore of an estuary (looking downstream) and higher salinities on the left. Pritchard has suggested that this diagonal orientation of isohalines is due to the earth's rotation. We have contended (Reid 1955) without denying the effects of Coriolis force, that in the case of East Bay currents from Galveston Bay (to the west) and Trinity Bay (to the north) and the configuration of Hanna Reef influenced the salinity pattern in East Bay to some extent.

The East Bay studies have been fruitful in demonstrating the rate and degree to which a pass such as Rollover Pass can influence the salinity of a bay. The salinity pattern observed during the 1955 study (Reid 1956) showed that within four months following the opening of the Pass the salinity in nearly three-fourths of the Bay was under the influence of the Pass, and that the salinity had at least doubled in approximately one-fourth of the Bay. Similarly, by the time of the third study (June, 1956) some five to six months following the erection of the barrier, only a small portion of the upper region of the Bay was affected by the Pass. With complete blocking of the Pass the salinity distribution would be expected to revert to that of an undisturbed estuarine body.

Temperature

Surface temperatures in the Bay during June, 1956, ranged from 26.5° to 31.0°C between the hours of 0800 and 1800. The warming process was rather rapid, with early morning temperatures of approximately 27.0°C reaching 31.0°C by 1530 hours. Cooling of the shallow estuary began at about 1630 hours and was dependent upon local meterological conditions.

Currents and tides

The considerable effect exerted by Rollover Pass on the hydrography of East Bay during the 1955 study has been discussed by Reid (1956). The 1956 study provided opportunity to observe the extent of the effect of the Pass in its partially blocked conditions. Thus, increased emphasis was placed on an investigation of tidal dynamics within the Bay with reference to the Pass and Galveston Bay, currents through Rollover Pass, and salinity exchange through the Pass. Although much of the data will require extensive study and a supplementary report, certain generalities can be presented here.

East Bay tides in 1956 were under the influence of the Galveston Bay system to a greater extent than in 1955. The general configuration of the isohalines (Fig. 2) indicates the Bayward movement of water masses through the Galveston Bay entrance. Salinity measurements at a shore station near Rollover further attested to the down-bay tidal flow (during the ebb) for most of the Bay. Salinity and current observations in Rollover Pass indicated that, during the summer season at least, the Pass served more as an outlet than an inlet. Certainly

the increased salinity in the upper portion of the Bay was the result of incursions of highly saline waters, but this may have been due to residual effects of more pronounced flow during the seasons prior to the present study and only weakly operating during the 1956 investigation. None of the numerous current determinations made in the Pass indicated strong inflow of Gulf water into the Bay. Even when the height differential between Gulf and Bay was sufficient to permit a Bayward flow, the measurable effects of current on the Bayward side of the highway bridge pilings diminished near the junction of Bay and Pass. Conversely, the outflow during the ebb of Gulf tides was usually much more conspicuous, with a considerable "head" being built up on the Bayward side of the pilings parallel to the bridge.

In attempting to correlate predicted tide ranges and times (Tide Tables, U.S.C. & G.).S with actual tide ranges and times, it was found that little agreement existed between the predicted daily ranges and observed ranges in East Bay. The predicted times of tide stages were essentially substantiated by field odservations. But where the predicted data inbicated ranges near 1.5 feet daily during the present study, tide gauge readings at Rollover revealed ranges of 0.8 foot or less. How constant this differential remains on an annual basis is, of course, not known.

BIOLOGIC ASPECTS OF EAST BAY, JUNE, 1956

Early consideration of raw data compiled during the present study pointed to considerable similarity between the structure of the fish population of East Bay during the summer investigations of 1954 and 1956. Thus, with full awareness of the shortcomings of the methods utilized, the time element involved in the study, and the biologic and behavioristic patterns of the species concerned, we shall develop the thesis that physically and biologically the Bay during the summer of 1956 was reverting to its original condition. The hypothesis that the fauna and ecology of East Bay were more similar to the original (1954) condition than the disturbed (1955) state rests on comparison of catch data for the three studies.

Three approaches to the problem are presented: (1) comparison of the abundance and distribution of those species that constituted the main mass of the trawl collections will show trends in population changes; (2) consideration of the species taken in small numbers but showing affinities for particular ecological conditions will give insight into the Bay as an environment; (3) analysis of the total catch for each of the three studies should reveal broad aspects of the population. The last-mentioned approach will be considered first.

Marked changes occurred in the abundance of fishes from 1954 to 1956. On the basis of trawl collections alone a significant decrease in total catch from 1954 to 1956 is noted (Table 1). Although the collecting effort was more than doubled (including the part-time use of a larger trawl) during the 1955 study, the average number of fish per collection in 1955 was less than half that of 1954. The average number per collection during the 1956 study was significantly higher than that in 1955, but did not closely approach the 1954 figure. Nevertheless, the 1956 data do suggest a trend toward the greater abundance of fishes existing in 1954. Table 2 presents a comparison of the total trawl catch and average number per collection of the most abundant fishes during each of the three investigations. In Table 3 similar presentation is made for the standard, 10-foot otter trawl (common to all three studies). All of these data relating to the fish population structure as reflected in the

TABLE 1. *Comparison of total catch by the various types of collecting gear and grand total for each of the East Bay studies, 1954, 1955, 1956*

Shown also is the average catch of fishes per trawl collection. Number of collections by each gear is given in parentheses.

Year	Trawl	Av./trawl coll.	Seine	Trammel net	Total
1954	19,055(32)	595.5	7,425(43)	371(6)	26,851
1955	18,400(78)	235.9	5,496(18)	77(5)	23,973
1956	17,769(61)	291.3	63,078*(11)	No collections	80,847*

* This catch is enlarged by what appeared to be an abnormally great number of menhaden, *Brevoortia patronus*, in the study area; nearly 63,000 individuals (enumerated volumetrically) were captured in eleven collections by means of a 40-foot minnow bag seine.

TABLE 2. *Comparison of the total trawl catch and average number of fishes per collection of the eleven most abundant species in 1954, 1955, and 1956*

Number of collections is shown in parentheses.

	Total catch			Average number per collection		
	1954 (32)	1955 (78)	1956 (61)	1954 (32)	1955 (78)	1956 (61)
Micropogon undulatus	9820	1965	7218	306.9	25.2	118.3
Leiostomus xanthurus	4750	1388	3421	148.4	17.8	56.1
Anchoa mitchilli diaphana	2332	11599	3546	72.9	148.7	58.1
Polydactylus octonemus	587	0	77	18.3	0	1.3
Cynoscion arenarius	487	387	1207	15.2	5.0	19.8
Brevoortia patronus	349	2069	799	10.9	26.5	13.1
Lagodon rhomboides	238	101	47	7.4	1.3	0.8
Galeichthys felis	175	191	366	5.5	2.4	6.0
Sphoeroides nephelus	128	106	540	4.0	1.3	8.8
Mugil cephalus	74	189	19	2.3	2.4	0.3
Citharichthys spilopterus	36	1	155	1.1	0	2.5
Total	18976	17996	17395	593.0	230.7	285.2

TABLE 3. *Comparison of the catch of the eight most abundant fish species in the ten-foot otter trawl collections in 1954, 1955, and 1956*

Number of collections is shown in parentheses.

	Total catch			Average number per collection		
	1954 (16)	1955 (40)	1956 (34)	1954 (16)	1955 (40)	1956 (34)
Micropogon undulatus	3072	446	1623	192.0	11.2	47.7
Leiostomus xanthurus	626	1014	681	39.1	25.4	20.0
Anchoa mitchilli diaphana	446+	4910	129	27.9+	122.8	3.8
Cynoscion arenarius	146	170	317	9.1	4.3	9.3
Polydactylus octonemus	103	0	9	6.4	0	0.3
Brevoortia patronus	81	747	218	5.1	18.7	6.4
Lagodon rhomboides	74	39	28	4.6	1.0	0.8
Galeichthys felis	70	17	165	4.4	0.4	4.9
Total	4618+	7343	3170	288.6+	183.6	93.2

trawl collections, considered generally or by species, strongly indicate a considerable similarity between the 1954 and 1956 data and a suggestion of a return of the Bay to its 1954 condition. Figure 3 portrays graphically the fluctuations in catch data relating to the dominant species in the trawl collections during the three investigations.

The main mass of the population as indicated by trawl collections comprised essentially the same species but in differing proportions during the three summers. During each of the investigations, seven or eight species constituted from 95 to 98 per cent of the catch. Consideration of this segment of the fish fauna in detail will further illuminate the problem concerning the direction of faunal succession. The species will be discussed in the order of abundance in the 1956 trawl collections.

The dominant fish species in East Bay

Micropogon undulatus (Linnaeus). Croaker. Specimens of this sciaenid were taken in each of the 61 trawl collections during the 1956 study. The catch made up 41 per cent of the aggregate fish catch by trawl in the Bay. The 1956 proportion of croaker was nearly thirty per cent higher than that of the preceding year and nine per cent less than in 1954. The data, however, indicate considerable similarity in abundance between 1956 and 1954. As indicated by seine collection data, the shallow-water population of croaker continued low during June, 1956; their numbers made up less than one per cent of the total seine collection. This is the same proportion experienced during the 1955 study and is to be compared with 18 per cent in 1954.

As observed during the two previous investigations, the greatest concentration of young croaker appeared to be in the upper portion of the Bay. The average number per trawl collection in that area in 1956 was 166.2, as compared with 68.3 in the middle region, and 61.5 in the lower Bay. The areal distribution of abundance was the same as in the two previous studies, and an interesting problem in abundance as related to salinity fluctuation, especially in the upper Bay, is posed. The conspicuous decline in numbers from 1954 to 1955 was accompanied by a substantial over-all increase in salinity, especially in the area of greater abundance (upper Bay) of the fish (Reid 1956). It would appear that if salinity is a critical factor the fish would have deserted the upper Bay, thereby possibly increasing the population density in another area. And since the salinity in the upper Bay remained high in 1956, the numbers there should have con-

tinued generally as in 1955. This, however, was apparently not the case. If salinity is a limiting factor, why did not the croaker move to regions of lower salinity more comparable to their original environment? Other factors must be considered. The bottom composition in the upper Bay is notably different from other Bay areas, being made up of loose, deep mud. Thus, it seems that the ecological affinity for the mud bottom (the food it offers?) prompts the young fish to remain in that area in the face of less favorable salinities. We use the term "less favorable" in the light of Gunter's (1945) data which indicate that in Copano and Aransas Bays, Texas, croaker are most abundant in salinities below 20‰.

Another consideration enters into the problem of fluctuation of the croaker population and may apply also to the population dynamics of other species. There is a possibility of mass emigration through Rollover Pass during 1955. However, this argument is weakened by the consistency of size ranges found during the three studies as well as the failure to catch the forms in large numbers in the Gulf.

Croaker caught during the 1956 study graded from 42 to 210 mm in length; most of the specimens ranged from 43 to 87 mm. The observed size range for 1956 was somewhat intermediate between that recorded in 1954 and in 1955 (Reid 1955b, 1956). The majority of the 1954 specimens ranged from approximately 38 to 80 mm (modal class: 60), and in 1955 the size range of most of the fish varied from 50 to 90 mm (modal class: 70).

Anchoa mitchilli diaphana Hildebrand. Bay Anchovy. The bay anchovy (Family Engraulidae) taken in 53 of 61 trawl collections constituted 19.9 per cent of the 1956 trawl catch. Eighty-seven specimens were caught in five of 11 seine collections in shallow water. The 1956 proportion of anchovy in the total trawl catch (20 per cent) represents a decline from the 63 per cent in 1955, although it is higher than the 12 per cent reported for 1954. The average number per collection for each of the three investigations (Table 2) similarly indicates the great increase in 1955 over 1954, and the subsequent decline in catch in 1956. It will be noted in Table 2 that the average catches for 1954 and 1956 are in closer agreement with each other than either is with that for 1955.

The spatial distribution of bay anchovy in the Bay followed a pattern similar to that of *M. undulatus*. In the upper Bay, the average number of anchovy per collection was 87.5, in the middle Bay 30.7, and in the lower Bay 18.6. These data are in agreement with those for 1955.

The length ranged from 14 to 60 mm during the 1956 study. This range, in general, compared favorably with size distributions during the previous investigations, except that fewer individuals larger than 50 mm were caught during the 1956 investigation.

Leiostomus xanthurus Lacépède. Spot. This sciaenid caught in 47 of the 61 trawl collections during 1956 constituted 19.2 per cent of the total trawl catch of fishes. Only four specimens were captured with the seine. The proportion of this species in the total trawl catch for 1956 represented an increase of eleven per cent over the previous year and is in close agreement with the catch for 1954, when spot made up 25 per cent of the total. Further evidence of increase in abundance of spot during 1956 over that reported for 1955 is shown in Table 2, where the average number per collection decreased from 1954 to 1955, but increased in 1956.

A marked gradient in density distribution was exhibited by spot during the 1956 study, with the center of abundance as with the preceding species, in the upper region of the Bay. The average number of fish per trawl collection in the upper Bay was 88.8, the middle Bay yielded 31.0, and 4.3 spot per collection were recorded in the lower Bay.

The over-all size range of *L. xanthurus* in 1956 was in agreement with the ranges recorded in 1954 and 1955. During the 1956 study the fish were 31 to 174 mm in length, the mode of the length-frequency distribution being 45 mm. Most of the specimens were 31 to 82 mm in length. One spot, 18 mm in length, was taken in a seine collection.

Cynoscion arenarius Ginsburg. Sand Trout. The combined trawl and seine

catch of this sciaenid[2] for 1956 amounted to 1,215 fish. Specimens were captured in 56 of 61 trawl collections and made up 6.8 per cent of the entire trawl catch. The average number per collection during the 1956 study represented a significant increase over that recorded during the 1955 study and a slight increase over the average catch during the 1954 investigation (Table 2). But again, the similarity between the 1954 data and those for 1956 is marked.

Distribution of *C. arenarius* in the Bay in 1956 revealed a somewhat erratic pattern, at least when compared with other sciaenids. Although the greatest abundance was found in the upper part of the Bay (average number per collection: 24.8), the decrease down the Bay was not so pronounced as in the instances of *L. xanthurus* and *M. undulatus*, and, further, the abundance in the lower region of the Bay was greater (average: 15.7 fish per collection) than in the middle area (13.2 individuals per collection).

Trout taken in the trawl during the 1956 study ranged from 20 to 230 mm in length, with a majority of the individuals being 35 to approximately 105 mm; a slight mode was indicated at 49 mm. The individuals making up the population sample during the 1956 study were markedly smaller than in 1954, and their sizes compared more favorably with the size range in 1955.

The summer food of *C. arenarius* in East Bay has been discussed by Reid (1955b) and by Reid, Inglis, and Hoese (1956). Their findings indicated the predominance of fishes in the diet of trout larger than 60 mm. Studies during the 1956 investigation agreed with the previous data and further emphasized the considerable importance of *Brevoortia patronus* in the ecology of the trout.

Brevoortia patronus Goode. Menhaden. Over 64,000 of this clupeid were taken in East Bay during the 1956 study. Of this number, nearly 63,000 were caught in seining operations in shallow water. Menhaden were captured in 26 of 61 trawl collections and their quantity comprised five per cent of the total trawl catch. When compared with the 1955 menhaden catch, which made up 11 per cent of the aggregate, it is apparent that the species was considerably more abundant in 1955 than in 1954. However, the 1956 catch approximated that reported for 1954, when *B. patronus* constituted two per cent of the total catch. Similarly, the average number of fish per collection for the three studies indicates a pronounced symmetrical fluctuation in population density (Fig. 3).

As indicated by trawl collections the greatest mass of the menhaden population was in the upper part of the Bay. Of the total trawl catch (799 individuals) only 56 specimens were caught in 17 collections in the middle part of the Bay, and three in 12 collections in the lower reaches. In an earlier paper Reid (1955b) pointed out that menhaden are "schooling fish" and that the possibility of collecting in a school at one time and missing a school another time tends to vitiate statistical treatment of the catch data.

The staggering number of menhaden in the littoral zone during the 1956 study is not explained. Collections were made in the same area in 1954 and 1955. The catch was 1,539 in 10 of 14 seine collections in the former year and 48 fish in 10 of 18 hauls in the latter year.

Individuals in the 1956 collections ranged in size from 18 to 61 mm; the modal class was 38 mm. The length range and length-frequency polygon compare favorably with the 1954 data. In 1955, more specimens larger than 60 mm appeared in the catch.

Sphoeroides nephelus (Goode and Bean). Swellfish. Swellfish (Family Tetraodontidae) were caught by all gear during the 1956 investigation. They occurred in 43 of 61 trawl collections, and 25 specimens were taken in eight of 11 seine hauls. Whereas the catch of swellfish in 1956 constituted three per cent of the total trawl collection, in each of the preceding two investigations the species entered the total at less than one per cent.

Swellfish were decidedly more abundant in the upper region of the Bay then in the other areas. There the average number of speci-

[2] The sea trouts are sometimes considered in the Otolithidae. We prefer to relate the genus to the family Sciaenidae.

mens per haul was 11.0, as compared with 2.9 and 3.3 in the middle and lower areas, respectively.

Although the 1956 specimens ranged from 13 to 89 mm in length, only 14 individuals were larger than 40 mm. Most of the fish were 25 to 39 mm in length.

Galeichthys felis (Linnaeus). Sea Catfish. The sea catfish occurred in 53 of 61 trawl collections during the 1956 investigation. None were caught in the seine. The trawl catch of sea catfish made up two per cent of the total fishes caught and constituted a greater proportion of the total catch in 1956 than during the previous studies. However, the population density appeared to be quite similar to that of 1954 (Table 2).

There was not a conspicuous difference in the distribution of *G. felis* in the various regions of the Bay during the 1956 study. The average number of individuals per collection in the upper Bay was 7.7, in the middle region 3.2, and in the lower Bay, 5.4. Data from the 1954 study indicated a greater affinity by this species for the upper portion of the Bay (Reid 1955b).

The size range of the fish was 45 to 284 mm. The small fish fell mostly into a group grading from 82 to 110 mm in length. The 1956 size range and length-frequency distribution of *G. felis* are in general agreement with size data obtained during the 1954 study, except for 12 individuals smaller than 80 mm caught during the present investigation.

Breeding by *G. felis* was in evidence during the 1956 study as in the two preceding investigations. Sixteen male catfish with mouth-borne eggs were caught; these fish ranged from 178 to 284 mm in length. The number of eggs carried by the males numbered from 8 to 36 and were in various stages of development. Breeding females ranged in size from 184 to 197 mm.

Less abundant fish species of ecological interest

Polydactylus octonemus (Girard). Eight-fingered Threadfin. During the 1954 investigation the catch of threadfin (Table 2) in 31 of 32 trawl collections made up three per cent of the total trawl catch. In spite of greatly increased fishing effort in 1955, none were taken. During the 1956 study threadfin were caught in 24 of 61 trawl hauls. The fish were caught with greater frequency in the upper region of the Bay; the average number of specimens per collection was 2.0 as compared with 0.3 in the middle Bay and 0.5 in the lower Bay.

Having been relatively common during the 1954 investigation, the absence of the fish during the 1955 study has been discussed in the 1955 report (Reid 1956). In both years the species was found in the Gulf, and during 1955 it had access to the bay through Rollover Pass. Although caught in small numbers, the presence of *P. octonemus* in the Bay during the 1956 study would seem to contribute positively to the thesis that the Bay was returning to its original condition.

Citharichthys spilopterus Günther. Whiff. This species was common in the Bay during the 1954 study, was rare in the 1955 collections, and reappeared in the 1956 catch (Table 2). In 1954, 21 of the trawl-caught fish were taken in one collection in the lower end of the Bay. More than doubled trawl collecting effort in 1955 over 1954 caught only one whiff.

During the 1956 study 158 *C. spilopterus* were caught. Three specimens were taken in two of 12 seine collections; the remainder was collected in 38 of 61 trawl hauls. On the basis of average number per trawl collection, the data indicate rather uniform distribution of the species in the deeper areas. Comparison of the seine collections for 1954 and 1956 suggests considerably fewer whiff in the shallow water in 1956.

Measurements of whiff collected in the trawl indicated a greater size range in 1956 (40 to 76 mm) than in 1954 (39 to 50 mm). The difference, however, may be attributable to a larger sample in 1956.

Lagodon rhomboides (Linnaeus). Pinfish. This species is noteworthy in that its density in the Bay had apparently declined continuously since the construction of the Pass (Table 2). A similar diminution in numbers in the littoral zone was indicated by seine collection data.

Stellifer lanceolatus (Holbrook). Star Drum. One hundred and eighty-seven specimens of the star drum were caught in

nine of 61 trawl collections during the 1956 investigation. The catch constituted slightly over one per cent of the aggregate trawl collection.

Trawl data point to a somewhat consistent increase in abundance of this species. The average number of star drum per collection was 0.2 in 1954, 0.6 in 1955, and 3.0 during the 1956 study.

The greatest abundance of this species during all three studies was found in the lower region of the Bay. In this area the salinity ranged roughly from 18 to 21‰ in 1956, from 12 to 16‰ in 1955, and from 16 to 19‰ during the 1954 study. The bottom in the area was sand and shell in certain localities and hard-packed mud and shell in others.

Star drum caught during the 1956 study ranged from 16 to 98 mm in length. Most of the specimens, however, fell into a somewhat bimodal distribution from 26 to 55 mm.

Fish species composition in East Bay, 1954–1956

During June, 1956, nearly 90,000 specimens of cartilaginous and bony fishes were collected. Represented in the 1956 collections were 44 species in 28 families. During the 1955 study, 24,000 specimens, including 51 species in 16 families, were caught. Approximately 27,000 fishes were taken during the investigation in 1954, and 50 species representing 30 families were present. In 1954, seven species made up 95 per cent of the catch. In 1955, the same number of species constituted 97 per cent of the total, and in 1956, eight species contributed to 98 per cent of the total collection.

Generally, the summer species composition in East Bay during the three investigations remained relatively stable. Thirty-one species were taken in trawl and/or seine collections throughout the series of studies. In addition to these, four species taken in trammel net collections in 1954 and 1955 failed to be recorded during the 1956 study, possibly because no collections were made with that gear. Nevertheless, these fishes—tripletail (*Lobotes surinamensis*), redfish (*Sciaenops ocellata*), spotted squeteague (*Cynoscion nebulosus*), and drum (*Pogonias cromis*)—were observed in sports catches in June, 1956, thus confirming their presence in the Bay. Therefore, the total number of species common to the three investigations becomes thirty-five. The species are listed in Table 4.

TABLE 4. *List of species present in East Bay during all three summer studies (1954–1956)*

Dasyatis sabina	*Hemicaranx amblyrhynchus*
Elops saurus	*Chloroscombrus chrysurus*
Harengula pensacolae pensacolae	*Lobotes surinamensis*
Brevoortia patronus	*Lagodon rhomboides*
Dorosoma cepedianum	*Archosargus oviceps*
Anchoa hepsetus	*Bairdiella chrysura*
Anchoa mitchilli diaphana	*Stellifer lanceolatus*
Synodus foetens	*Sciaenops ocellelata*
Bagre marinus	*Leiostomus xanthurus*
Galeichthys felis	*Micropogon undulatus*
Fundulus grandis grandis	*Pogonias cromis*
Cyprinodon variegatus variegatus	*Cynoscion arenarius*
Membras martinica vagrans	*Cynoscion nebulosus*
Mugil cephalus	*Chaetodipterus faber*
Mugil curema	*Citharichthys spilopterus*
Trichiurus lepturus	*Paralichthys lethostigma*
Peprilus paru	*Achirus lineatus*
	Sphoeroides nephelus

The presence or absence of species during one or another of the studies have implications relating to environmental factors, migration tendencies, or collecting methods. In some instances it is difficult to determine which of these aspects is most applicable to the described condition. But where some knowledge of the ecology and distribution, migrations, and behavior exists, we feel more at liberty to offer some explanation for certain phenomena.

Three species reported during the 1954 study were not collected subsequently. Two specimens of the spot-finned shark (*Carcharhinus limbatus*) were caught in the trammel net during that study. Although not taken in trammel net collections in 1955, or noted in 1956, sight records indicated that this shark inhabits the Bay. The spotted gar (*Lepisosteus productus*) was also caught by trammel net during the first study but was not reported in later investigations. *Mollienesia latipinna*, the sailfin,

was present (but uncommon) in 1954, but not during later investigations.

Ten species were recorded for the first time during the 1955 study following the opening of Rollover Pass. Of these, five species were not noted in 1954, *i.e.*, they appeared in the collections only during the 1955 study.

Alligator gar (*Lepisosteus spatula*) were caught in trammel net collections during the 1955 period; there is little doubt that the species was present in the Bay at all times.

Although abundant in the shallow Gulf during all three studies and present in the Pass proper in 1955 and 1956, young pompano (*Trachinotus carolinus*) were collected in the Bay only in 1955. Since this species is usually associated with higher salinities, it is reasonable to assume that access to, and increased salinity in, the bay accounted for their presence there in 1955. In June, 1956, the salinity of the Bay waters in the vicinity of the Pass remained high. However, passage of the fish through the pass might have been inhibited by the barrier-like pilings[3] or the configuration of the channel and rate of current flow, all of which had been considerably modified from the 1955 condition.

Three banded croaker (*Larimus fasciatus*), one whiting (*Menticirrhus littoralis*), and one spiny boxfish (*Chilomycterus schoepfi*) were likewise collected only during the 1955 study. It is likely that the salinity-access relationship discussed above also applies in these cases. However, the small samples weaken the argument.

Five of the ten species recorded first in 1955 were repeated in 1956, although in reduced numbers in all but one instance. Several specimens of *Adinia xenica*, a small cyprinodont, were caught in shallow water in 1955; one was taken in the same area during June, 1956. Young mackerel (*Scomberomerus maculatus*), normally an inhabitant of waters of high salinity, were captured by trawl and seine in 1955; one was caught by seining during the 1956 investigation. The whiting (*Menticirrhus americanus*) was represented in the 1955 collection by 10 specimens; 20 were caught in 1956. Three goby (*Gobionellus boleosoma*) were captured by seining in 1955; one was caught in 1956.

Excluding the previously discussed species caught by trammel net, six forms present in the collections of 1954 and 1955 were not taken during the 1956 study. They are as follows, with no explanation for their nonoccurrence other than the small numbers in which they were caught in the previous collections:

Signalosa petenensis
Myrophis punctatus
Syngnathus louisianae
Fundulus similis
Menidia beryllina peninsulae
Caranx hippos

The 1956 study of East Bay added only one new species record to the extant list. This is an as yet unidentified serranid represented by one young individual. Sports fishermen along the pass landed fair numbers of *Mystriophis mordax*, an ophichthid eel. This species was not observed the previous year among the discarded fishes on the shores of the Pass. It is not known to occur in the Bay.

As previously implied, the basic practical consideration in the construction of passes such as Rollover Pass is the improvement of sport fishing. In this region the spotted squeteague (known locally as speckled trout), *Cynoscion nebulosus*, is probably the most highly prized of the inshore sport fishes. Thus the effects of Rollover Pass on the abundance and size of squeteague were of concern to our study.

In an earlier publication we have discussed the apparent absence of young squeteague in East Bay (Reid 1956). None was caught during the 1956 study. Our data strongly indicate that East Bay does not serve as a nursery for this species.

However, the population of adult squeteague in the Bay during the summers of

[3] Unpublished data from experiments conducted by the author on the behavior of certain marine fishes (Atlantic croaker, spot, gray weakfish, silver perch, and menhaden) before various net and rope barriers indicated a reluctance of the fishes to pass through the barriers. This reaction of fishes is a basic consideration in the use of a hedging, or leader, in pound net construction [Reid. 1955. The pound net fishery in Virginia. Comm. Fish. Rev., **17**(5): 1–15.]

1955 and 1956 appeared to be considerably greater than previously. Indeed, observations of sports fishing effort and catch (the basis for all our conclusions concerning this fish) point to increased numbers and larger sizes of squeteague. Certainly this particular aim of the Pass construction was realized.

Invertebrates in the Bay ecology

It is singularly interesting that the fluctuations in population density of two species of shrimp should so closely parallel those exhibited by several fish species as shown in Figure 3. Figure 4 depicts the average number of brown shrimp (*Penaeus aztecus*) and white shrimp (*Penaeus setiferus*) per trawl collection during each of the three studies.

During the 1954 study, 5,579 *P. aztecus* were caught in 31 of 32 trawl collections, and 2,584 were taken by seining. The average number of brown shrimp per trawl collection was 174.3 (the figure 115 per collection [Reid 1955a: 341] is in error). The specimens ranged from 32 to 89 mm in length. During the same investigation 14 *P. setiferus*, 108 to 143 mm in length, were captured. Thus the total trawl catch of both species during the 1954 study was 5,593 of which *P. aztecus* made up over 99 per cent.

During the second study, June, 1955, the total trawl catch of the two species was 4,147. Of the total, 1,713 *P. aztecus* (41 per cent) were caught in 71 of 78 trawl collections for an average of 21.9 per collection. The catch of *P. setiferus* in the same period amounted to 2,434 individuals (59 per cent) taken in 43 of the 78 trawl collections, with an average frequency of 31.2 examples per collection. *P. aztecus* caught during the 1955 study were 19 to 136 mm in length. *P. setiferus* ranged in length from 21 to 124 mm.

In the course of the 1956 investigation, 7,183 *P. aztecus* (99.8 per cent of the total of the two species) were taken in 60 of 61 trawl collections with an average frequency of 117.7 individuals per haul. Thirteen *P. setiferus* were caught in six of 61 trawl collections. Analysis of the trawl collection data revealed a greater abundance of *P. aztecus* in the upper part of the Bay; the average number per collection in that region was 155.9, as compared with 66.6 in the middle Bay, and 86.2 shrimp per haul in the lower reaches of the Bay. These findings agree essentially with the data for 1955, but not with the figures for 1954. During the 1954 study *P. aztecus* was more abundant in the lower two-thirds of the Bay than in the upper area.

Brown shrimp in the 1956 trawl catch were from 28 to 141 mm in length. Most of the individuals in the size distribution ranged from 36 to 100 mm with a mode at 65 mm. The general configuration of the length-frequency distribution for 1956 is in close agreement with the figures for 1954 (Reid 1955a) and for 1955 (Reid 1956).

Xiphopeneus krøyeri (Heller). Sea Bob. This species, not found in East Bay during the 1954 study, appeared in the 1955 catch; 112 specimens were taken in three of 78 trawl collections. The catch was made in the upper part of the Bay in the area of highest salinity (Reid 1956). This was also the region nearest Rollover Pass.

Eighteen sea bob were caught during the 1956 study. All were taken in one collection in the extreme lower end of the Bay along the north shore. The salinity at the time of capture was 19.4‰.

The size range of *X. krøyeri* caught during the 1956 investigation was 63 to 93 mm. This compares favorably with the range observed in 1955.

Reid (1956) has briefly discussed the presence of the sea bob in East Bay in relation to salinity.

Callinectes sapidus Rathbun. Blue Crab. During the 1956 investigation, 358 blue crabs were caught in 49 of 61 trawl collections with an average frequency of 5.8 per haul. These data indicate a greater population density of the species in 1956 than previously. In 1954, the catch was 95 individuals in 19 of 32 trawl collections; 120 crabs were caught in 30 of 78 hauls in 1955.

The size range (carapace width) of *C. sapidus* caught during the 1956 investigation was in general agreement with size data obtained during the previous studies.

Four ovigerous females, 160 to 184 mm

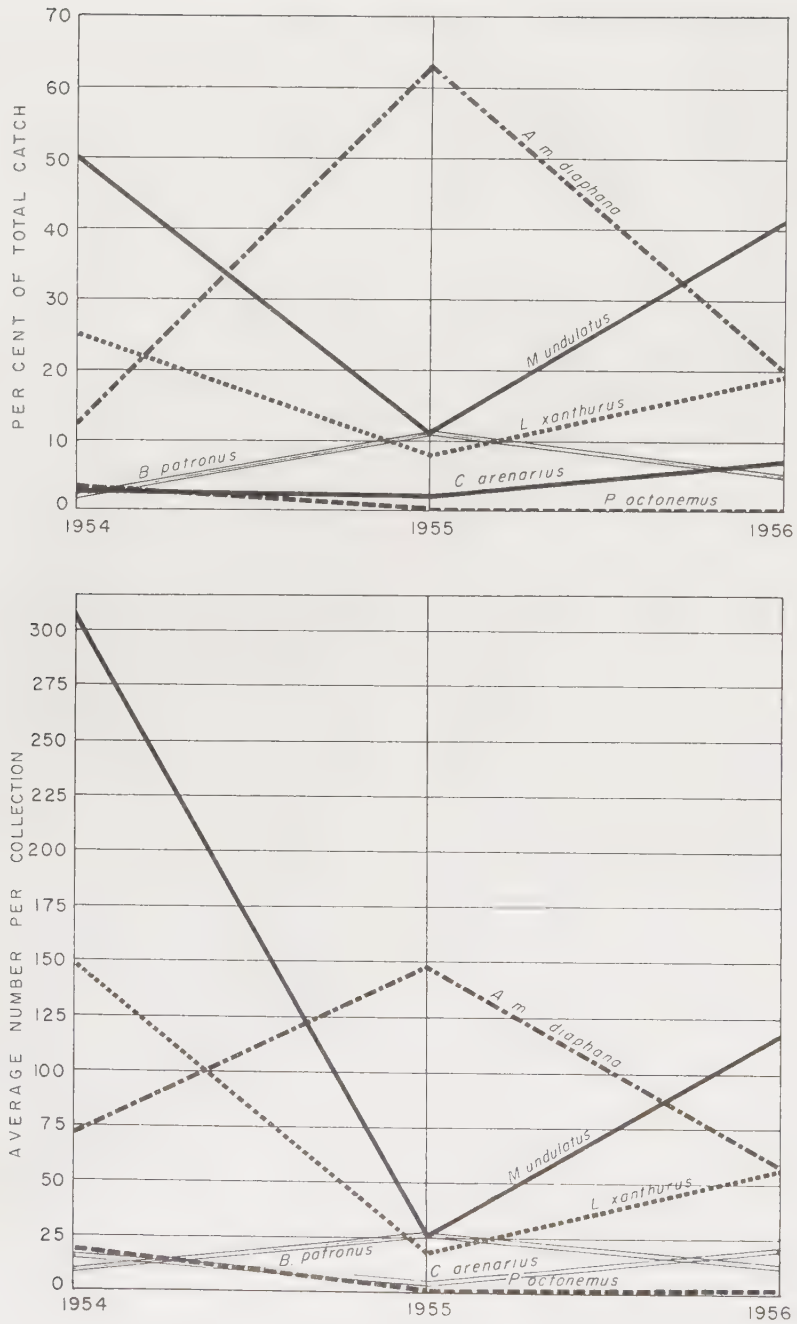

FIG. 3. Fluctuations in population density of dominant fish species, 1954–56, as indicated by trawl collections. *Above*: Graph based on per cent of each species in total catch. *Below*: Graph based on average number of each species per trawl collection.

(carapace width), were caught during the 1956 study. Three were taken in the lowermost part of the Bay in salinities from 20.0 to 21.0‰; the fourth individual was caught toward the upper part of the Bay (salinity: 16.2‰). Three of the females were carrying yellow egg-masses ("sponges"), while one bore an orange sponge indicating more advanced development of the eggs.

Lolliguncula cf. *brevis* (Blainville). Squid.

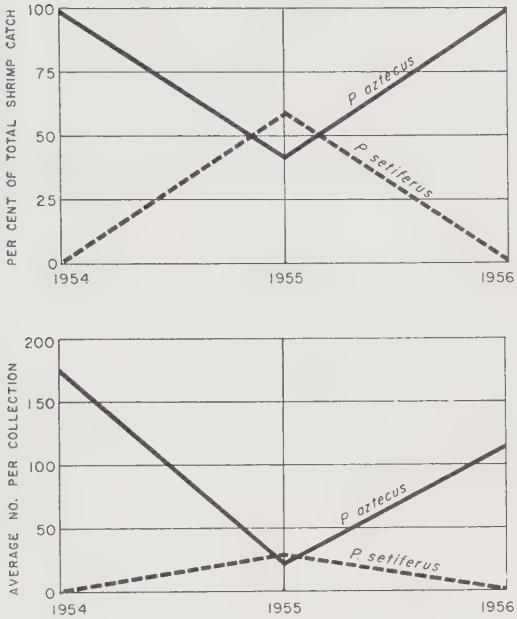

FIG. 4. Fluctuations in trawl catch of two shrimp species, 1954–1956. *Above:* Figure based on percent of total catch. *Below:* Figure based on average number of each species per collection

East Bay data indicated a continuous decline in the catch of squid during the three investigations. The average frequency in 1954 was 2.1 individuals per collection, 0.5 in 1955, and 0.4 in 1956.

It appeared during the first and second studies that the distribution of squid in the Bay might be correlated with salinity in that the animal was more abundant during the 1954 study in the lower part of the Bay, and in 1955, in the upper region. Those areas were the zones of highest salinity during each of the investigations. However, during the 1956 study squid were again found to be most numerous in the middle and lower Bay in intermediate salinities, apparently shunning the upper Bay where the most saline waters occurred.

The coelenterates, ctenophores, and certain other invertebrates of East Bay during the summer have been discussed previously (Reid 1955a, 1956). The same forms were present during the 1956 study, and no conspicuous changes in abundance or distribution of the forms were observed.

It appeared from general observations that the oyster (*Crassostrea virginica*) was more abundant during the 1956 study than previously. Superficial examination of individuals also suggested larger size and better condition than was noted during the earlier investigations. Concentrated research on oyster biology and development in relation to the salinity fluctuations in East Bay is needed.

SUMMARY

1. Three summer investigations of the biology and ecology of East Bay, Texas, have been accomplished. These studies were undertaken to determine the effects of introducing Gulf of Mexico waters of high salinity into the estuary as a consequence of the excavation of Rollover Pass near the head of the Bay.

2. The first study was made during the summer of 1954, prior to cutting the Pass. The second investigation, during the summer of 1955, took place following the construction of the Pass when there was great interchange between Gulf and Bay through the Pass. The 1956 summer study was made at a time when the effects of the Pass had been considerably inhibited by erosion control barriers placed across the channel.

3. Hydrographic data obtained during the 1956 study are presented, and point to the lessened role of the Pass in contributing Gulf water to the Bay. The salinity distribution throughout most of the estuary closely resembled that of the 1954 undisturbed condition.

4. The composition of the 1956 fish population resembled more closely that of 1954 than that of 1955, suggesting a correlation with salinity, and a return to the undisturbed condition.

5. The population fluctuation of two shrimp species appeared to follow that of the dominant fishes.

6. The presence or absence of certain forms and modification of numbers of others as related to changes in the hydroclimate are discussed.

7. Catch data revealing the relative abundance of the larger fishes and invertebrates essentially substantiate a thesis that the Bay was, in 1956, reverting to its 1954 state.

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Some Observations on the Stratification of Lake Victoria

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ABSTRACT

The stratification of temperature and oxygen is described for the open water of Lake Victoria during March May 1956. The results generally conform to an outline of seasonal changes previously recorded for 1952–4. At the period of strongest stratification a shallow and almost deoxygenated lower layer is marked off by a distinct but deep thermal discontinuity.

The vertical distribution of the planktonic algae during the period of strong thermal stratification is described, with the effects of a later change to more isothermal conditions. Some conspicuous differences between the various species are recorded and classified; thus maximum densities of different species are variously found in upper, middle, and lower layers. These differences are considered to arise mainly from different sinking rates and capacities to withstand conditions in a deoxygenated lower layer. The relation between algal distribution and the photosynthetic zone is briefly discussed.

INTRODUCTION

There is little detailed knowledge of stratification in large tropical lakes. As regards changes of thermal stratification Lake Victoria would appear to be the best known example, following observations in 1927–8 by Graham (1929) and Worthington (1930) and in 1952–4 by Fish (1957). Worthington (1930) concluded that the central open waters of the lake were permanently stratified, but with only a small temperature gradient and lacking a marked discontinuity or thermocline. His records, however, extended only over a period of five months, and did not include a prolonged series of observations at a single station. Such a series, extending over 18 months, was undertaken by Fish (1957). It showed thermal stratification to be either absent or extremely slight from mid-May to mid-August, the coolest season, after which a definite stratification developed. The latter became most marked between March and mid-May, when a conspicuous discontinuity layer was present within the depth range 40–60 m. This discontinuity behaved as a strong barrier to vertical transport, and near the end of its existence marked off a shallow and almost deoxygenated lower layer. The temperature gradients involved were small when compared with the thermoclines of temperate lakes, but the corresponding density gradients were less divergent owing to the high temperatures of Lake Victoria (cf. p. 220). The discontinuity, and also isotherms elsewhere, showed vertical oscillations of considerable amplitude due to direct wind effects and an internal ("temperature") seiche in the lake basin.

This paper describes some aspects of stratification between March and May 1956. They were studied for comparison with conditions found by Fish in the same season of 1953, when the strongest stratification within the yearly changes was encountered. In addition, a more detailed study was made of the vertical distribution of the phytoplankton within this particularly interesting season, and a comparison given between that distribution and the extent of the photosynthetic zone.

This work was carried out from the laboratory of the East African Fisheries Research Organization by the kind invitation of the Director, Mr. R. S. A. Beauchamp, to whom I am indebted for various information. My thanks are also due to Mr. J. D. Roberts for much help when on the lake, to Dr. G. R. Fish for permission to reproduce some of his data, and to Drs. J. W. G. Lund and C. H. Mortimer for criticism of the manuscript.

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METHODS

Records of stratification during 1956 were made at the same station as in 1952–4, in an area over deep water (usually 60–65 m) at 0° 08′ S, 33° 03′ E. Temperature was measured and water samples collected with the apparatus used by Fish (1957)—a Ruttner water-sampler fitted with a mercury thermometer. The latter was read to 0.05°C. Dissolved oxygen was determined by the unmodified Winkler method, and a slight underestimation of oxygen by interference from reducing substances in the more deoxygenated water is therefore possible. I am indebted to Mr. R. S. A. Beauchamp for the earlier estimations (1–21 March), which were performed colorimetrically using the B. D. H. "Nesslerizer"; the calibration of the latter was checked by the normal iodine titration. Although the results obtained colorimetrically were less accurate than those obtained by titration, they were adequate for determining the main features of the stratification. The thiosulphate employed in titrations was standardized against decinormal potassium dichromate solution.

Counts of planktonic algae (of healthy appearance) were made by the sedimentation technique of Utermöhl (1931) and Lund (1949). The sample volumes used, which ranged up to 150 ml, yielded 50–150 individuals of the more abundant species and 30–160 individuals of the minor species at the more populated depths.

Light penetration was measured by an underwater photometer of conventional design (cf. Atkins, Clarke, Pettersson, Poole, Utterback, and Ångström 1938), with opal glass and Schott green (VG9) filter. Measurements influenced by non-linearity in the photo-cell response at high light intensities were avoided.

STRATIFICATION OF TEMPERATURE AND OXYGEN

The seasonal changes in thermal stratification found by Fish (1957) and already outlined are best displayed by isotherms drawn on a depth-time diagram (Fig. 1). The isotherms often exhibit large vertical oscillations, which Fish has shown to result from direct wind effects and an internal seiche in the lake basin. For general descriptions of these phenomena the reader is referred to Ruttner (1953: 44–6) and Mortimer (1952, 1953). At any one station they cause the thicknesses of the warmer and cooler layers to alternately expand and contract. These movements may be large enough to cause the full depth to be temporarily occupied by water of one type, then giving the impression of an absence of thermal stratification in the lake. Fish demonstrated such a situation to have arisen in January 1953 (see Fig. 1).

Against this background may be viewed the present records of changes in temperature and dissolved oxygen. The variables are shown by isotherms or isopleths (lines of

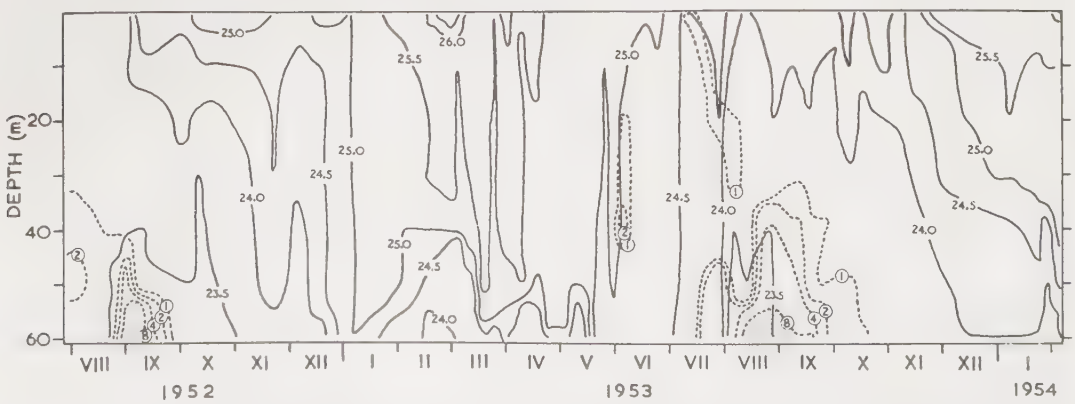

FIG. 1. Depth-time diagram showing changes in the thermal stratification, and the *Melosira* population, during 1952–4. Isotherms (solid lines) are given at intervals of 0.5°C. Isopleths of *Melosira* density (broken lines) show densities (ringed) of 1, 2, 4, and 8 cells per 0.01 ml. Areas with densities exceeding 8 cells per 0.01 ml. are stippled. The data are those of Dr. G. R. Fish and are reproduced here by kind permission.

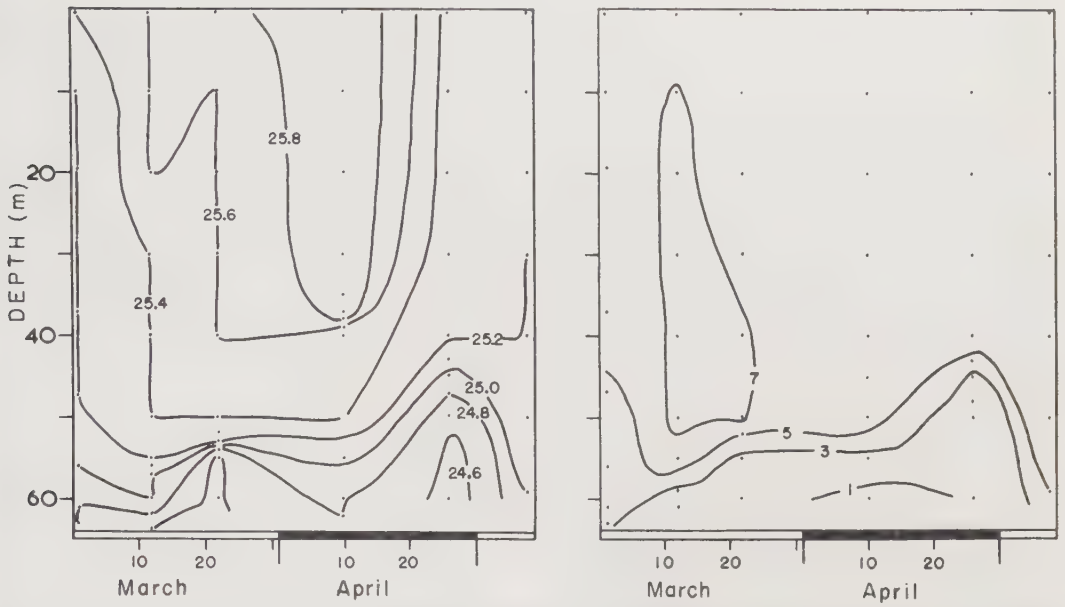

FIG. 2. Depth-time diagrams showing changes in the stratification of temperature (left) and dissolved oxygen (right) during March–May 1956. Temperature is given in °C and oxygen in mg/L.

equal concentration) on depth-time diagrams (Fig. 2). Bunching of the isotherms or isopleths indicates steep gradients in time or space whereas wide spacing indicates shallow gradients. Comparison of Figures 1 and 2 showed that the general thermal structure was similar in the corresponding periods of 1953 and 1956, with a discontinuity layer in the lower region (40–60 m). Vertical oscillation of this layer is less evident in 1956 than in 1953, but details may well have been lost by the relatively infrequent observations.

Some other minor differences are apparent between 1953 and 1956, and probably arise from small differences of timing. The very weak stratification on 1 March 1956 probably resulted from displacement of isotherms by direct wind action and the internal seiche, such as occurred in January 1953. Warming of the upper layers reached a maximum somewhat later in 1956 than in 1953.

The distribution of dissolved oxygen in 1956 (Fig. 2) was generally similar to that described by Fish (1957) for 1953, with considerable depletion in the lower layers. In 1953 the depletion during this season was the greatest recorded during the year. Changes in the depth of the discontinuity layer, mentioned above, can be traced in the oxygen isopleths. The concentration of oxygen in the upper half of the water column, during the period (10 April to 8 May) in which more accurate measurements by titration were made, lay between 92 and 100% of the revised saturation values of Truesdale, Downing, and Lowden corrected for altitude (see Mortimer 1956).

Thermal stratification during 1953 was lost before 16 May, and an appreciable redevelopment did not occur until August. In 1956 most of the thermal stratification disappeared a little earlier, between 26 April and 8 May, with a corresponding decrease in the stratification of oxygen. Tilting of isotherms by the internal seiche may have played some part in the disappearence of the cooler bottom layer, but a progressive cooling of the upper layers was certainly involved. If the effect of the seiche were the more important, the loss of stratification would not extend to the open lake as a whole. Further observations to clarify this point unfortunately were not possible.

STRATIFICATION OF THE PHYTOPLANKTON

The first records of vertical distribution of algae in the open lake are qualitive

(Worthington 1930 Table 7, and Bachmann 1933, both based on the same collections). The quantitative data of Fish (1957) concern the distribution of certain diatoms (*Melosira* and *Nitzschia*) whose densities were often too low for accurate enumeration by his method during the period of strongest thermal stratification. Estimations from larger samples are given below, which enable a comparison of distributions for the various species and for the same species under conditions of strong and weak thermal stratification.

During relatively strong thermal stratification, 26 April 1956

The vertical distribution of five of the predominant species was followed (Fig. 3); the diffluent colonies of the sixth, *Aphanocapsa delicatissima* W. et G. S. West, were difficult to count. A thermal discontinuity between 40 and 50 m separated an almost uniform and well oxygenated upper layer from a lower layer poor in oxygen.

Three types of algal distribution can be distinguished. In the first, shown by the bluegreen algae *Lyngbya circumcreta* G. S. West and *Aphanocapsa elachista* W. et G. S. West, maximum densities are found in the upper layers (0–20 m), with a decline to very small numbers in the lower layers (50–60 m). In the second, the unicellular diatoms *Cyclotella kutzingiana* Thwaites and *Nitzschia acicularis* W. Smith are most abundant in the middle layers though also frequent above; again numbers are much smaller in the lower layers. The third type, that of the filamentous diatom *Melosira nyassensis* var. *victoriae* O. Müller, is very different, for the greatest numbers are found in the lowest layers and around the thermal discontinuity with very few filaments above. Most of the cells in the lower layers appeared very healthy despite the deoxygenation. The records of Fish (1957) for *Melosira* are similar.

The three types of distribution cannot arise directly from the growth of the species showing various optima associated with different depths, as in all cases appreciable photosynthesis was only possible in the upper layers (0–20 m; see p. 219). They can be explained by different rates of sinking and differing degrees of resistance to the conditions in the nearly deoxygenated lower layer (cf. reviews by Ruttner 1914 and Gessner 1955: 378–439). If the sinking rate progressively increased from the *Aphanocapsa* and *Lyngbya*, to the *Cyclotella* and *Nitzschia*, and finally to the *Melosira*, the observed distributions would result, provided that only the *Melosira* could withstand conditions in the lower layer. Lund (1954) has shown that another *Melosira* species could survive for long periods in the deoxygenated hypolimnia of several English lakes, under con-

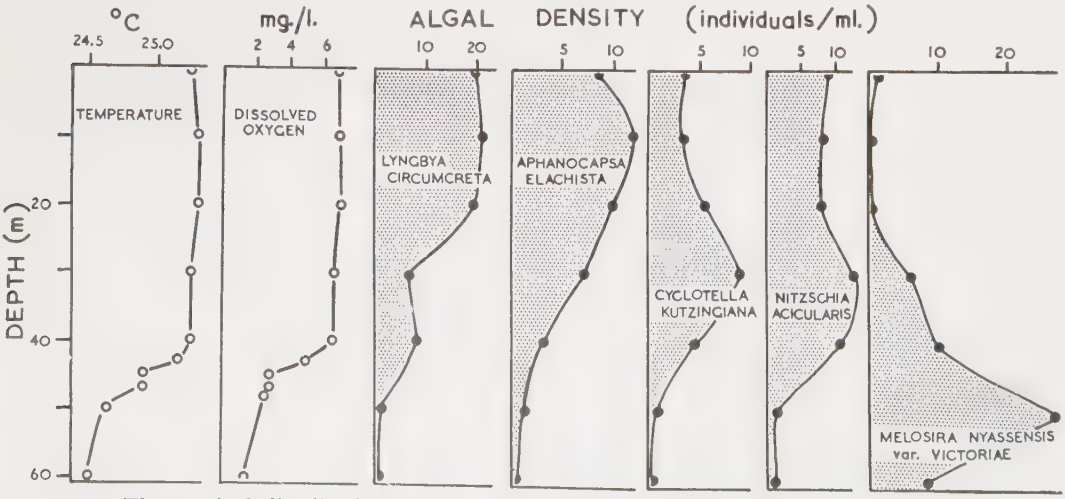

FIG. 3. The vertical distribution of temperature, oxygen, and major components of the phytoplankton on 26 April 1956. "Individuals" of algae are cells except for *Lyngbya* (coil-turns) and *Aphanocapsa* (colonies).

ditions in which many associated algae were killed. Similar results were obtained by Fish (1957) for the *Melosira* from Lake Victoria. Lund also demonstrated that his *Melosira* populations had a high sinking rate, a feature which dominated their depth distribution. For the algae studied here, some rough observations on sedimentation in a counting tube (cf. Fritz 1935) support the assumptions given above about their relative sinking rates.

During very weak thermal stratification, 8 May 1956

Most of the thermal stratification observed on 26 April had disappeared by 8 May (Figs. 2, 4) with several effects on the algal distribution (compare Figs. 3 and 4). The species previously almost absent from the lowermost layer appear there in some numbers after the disappearance of the discontinuity layer, thus giving a more even distribution of these populations. Relatively large numbers of the diatoms *Cyclotella* and *Nitzschia* appear in the bottom layer (50–60 m), whereas maxima of the bluegreen algae (*Lyngbya*, and particularly *Aphanocapsa*) are still at higher levels. This difference would be expected if the bluegreen algae have the lower sinking rate, as was previously postulated.

As before, *Melosira* presents a special case. Greatest numbers of this diatom are still present in the lower layers, but increased mixing following the disappearance of the thermal discontinuity has caused the transport of more cells into the upper layers. An increase has also occurred in the number of cells near the mud surface, which has probably been augmented from cells previously resting on that surface. Similar movements by another *Melosira* species has been described by Lund (1954, 1955). On the evidence then available (Anon 1952), Lund (1954) suggested that the behavior of the *Melosira* population in a channel of Lake Victoria resembled in several respects the behavior he found in the English lakes. Rapid sinking of cells during thermal stratification, and their subsequent transport upwards during periods of isothermal mixing, were events that decisively determined the period available for growth in the illuminated upper layers.

For the open water of Lake Victoria, confirmation is provided by a more detailed later description of seasonal changes in the *Melosira* population (Fish 1957). In Figure 1 Fish's data are used to plot isopleths of the density of *Melosira* in the same diagram as was used to show temperature changes. In both 1952 and 1953 the greatest numbers of *Melosira* appeared about the end of the period of near-isothermal mixing, and declined on the development of stronger thermal stratification. Further discussion of these changes can be found in Fish's paper.

The algae so far discussed are (with *Aphanocapsa delicatissima*) the most abundant species present, reaching densities of

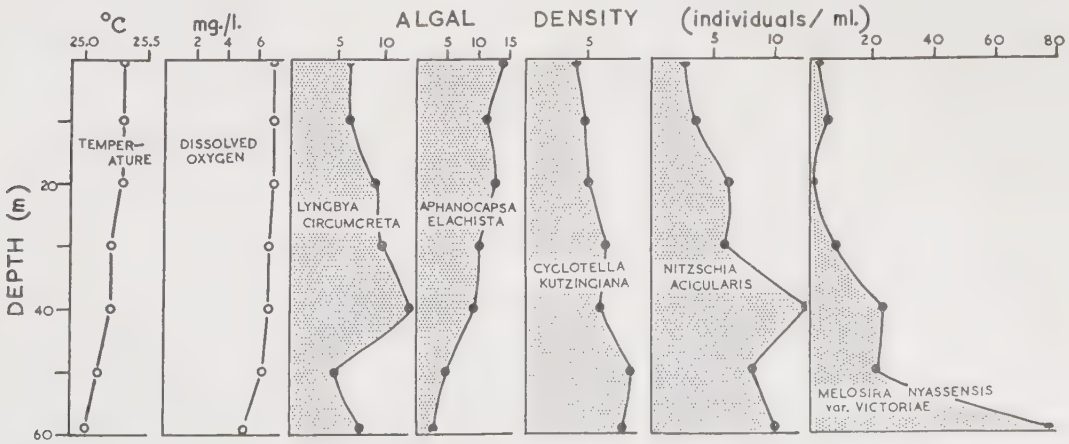

FIG. 4. The vertical distribution of temperature, oxygen, and major components of the phytoplankton on 8 May 1956. "Individuals" of algae are as in Figure 3.

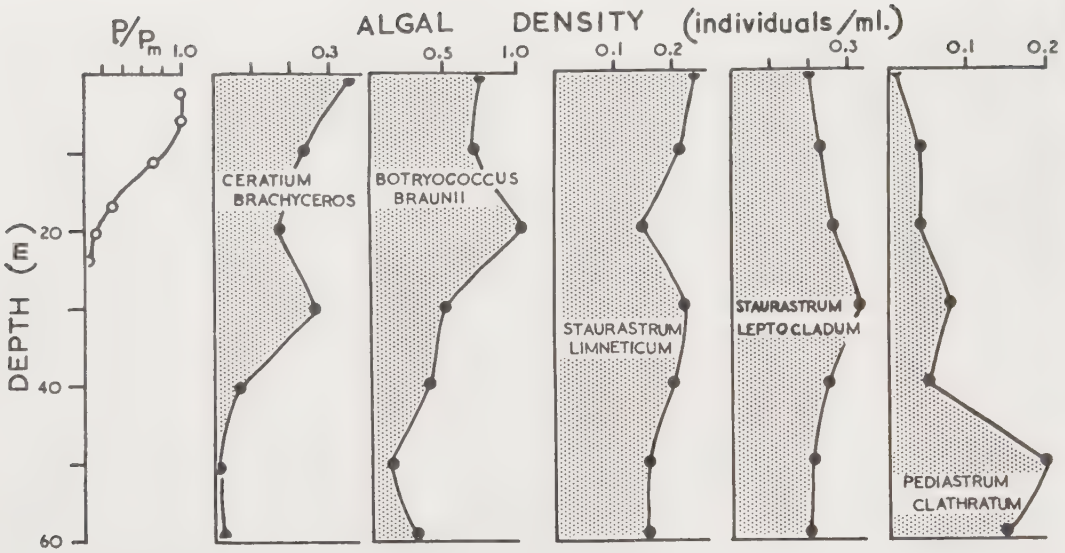

FIG. 5. The vertical distribution of some minor components of the phytoplankton on 8 May 1956. "Individuals" are cells except for *Botryococcus* and *Pediastrum* (colonies). An estimated depth profile of photosynthesis is included; photosynthetic rate is shown as a fraction (P/P_m) of the maximum rate (P_m).

8–78 individuals per ml. The depth distribution of some minor constituents, with maximum densities of 0.2–1.0 individuals per ml, was also followed during the almost isothermal conditions of 8 May 1956. The distributions shown (Fig. 5) are remarkably diverse considering the absence of any marked thermal stratification in the water column. The colonial green alga *Botryococcus braunii* Kütz. and the dinoflagellate *Ceratium brachyceros* v. Daday show maxima in the upper layers (cf. *Aphanocapsa*, Fig. 4), whereas *Pediastrum clathratum* (Schröt.) Lemm. has a distinct deep maximum. An intermediate pattern is shown by the desmids *Staurastrum limneticum* Schmidle and *S. leptocladum* Nordst. f. *africanum* G. S. West, which are distributed almost uniformly with depth.

These species differences are probably also the result of differences in sinking rate, without the added factor of resistance to conditions in the deoxygenated lower layer. Thus *Botryococcus* is well known as a remarkably buoyant alga. The *Ceratium* is likely to maintain its upper maximum by means of its motility; diurnal migrations in depth have been described for this genus both in lakes (Thienemann 1917: data reproduced in Utermöhl 1925: 204–5) and in the sea (Hasle 1954).

The types of distribution can be conveniently summarized in the four classes below. *Lyngbya circumcreta* is here omitted, as its behavior resembles that of *Aphanocapsa elachista* in the stronger thermal stratification but not in the weak stratification.

Class I. Maxima in the upper layers (0–30 m).....
Ceratium brachyceros
Botryococcus braunii
Aphanocapsa elachista

Class II. Nearly uniform distribution.....
Staurastrum limneticum
S. leptocladum f. *africanum*

Class III. Weak maxima in the lower layers (30–60 m).....
Cyclotella kutzingiana
Nitzschia acicularis

Class IV. Strong maxima in the lower layers (30–60 m).....
Pediastrum clathratum
Melosira nyassensis var. *victoriae*

PHYTOPLANKTON STRATIFICATION AND THE PHOTOSYNTHETIC ZONE

Much of the significance of the vertical distribution patterns of the phytoplankton lies in their relation to the euphotic zone, within which light intensity is sufficient for

appreciable photosynthesis. The thickness of this zone can be estimated either directly from measurements of the variation of photosynthetic rate with depth, or indirectly from measurements of light penetration. The latter measurements, but not the former, were made at the station concerned. They are employed below to adapt measurements of photosynthesis made elsewhere in Lake Victoria (Talling 1957c) to illustrate the photosynthetic zone at this station. The results are compared with more direct measurements of photosynthesis made here in 1953 by Levring and Fish (1956), who used both natural and cultured populations of algae in samples suspended at various depths.

The method used here is based upon a previous study of phytoplankton photosynthesis in several English lakes (Talling 1957a, b). It was shown that the vertical extent of a curve describing the variation of photosynthetic rate with depth (depth profile) in a lake water was inversely proportional to the minimum, over the visible spectrum, of the vertical extinction coefficients of the lake water. Such coefficients are illustrated, for example, by Sverdrup, Johnson, and Fleming (1942: Fig. 20). Consequently, if a depth profile is known for a lake water with a known minimum vertical extinction coefficient, it is possible to transform the depth scale so as to estimate the corresponding profile for a second lake water whose minimum coefficient is also known. This transformation assumes that other factors affecting the depth profile, such as the surface light intensity and temperature, remain essentially unaltered.

The transformation has here been applied to a depth profile of photosynthesis recorded on 1 April 1956 in Pilkington Bay (0° 17′ N, 33° 20′ E) on Lake Victoria. The material used was a net collection of *Melosira nyassensis* var. *victoriae* from the Bay; further details of this work will be published elsewhere (Talling 1957c). Minimum vertical extinction coefficients in the Bay and open lake station were respectively 0.64 and 0.18 per m. Consequently the depth scale of the profile measured in the Bay has been multiplied by a factor of 3.6 (= 0.64/0.18) to represent conditions in the clearer water of the open lake. The resulting profile may undergo some modification in relation to other algal species or other conditions of surface illumination. Such modification is unlikely to be large for average daily conditions, as the exponential nature of light diminution with depth causes the depth of the photosynthetic zone to be primarily controlled by the optical properties of the water. Illustrative examples are given by Talling (1957a, b) and for East African lakes including Lake Victoria by Levring and Fish (1956).

The transformed depth profile of photosynthesis (Fig. 5) indicates a photosynthetic zone of about 20 m depth, or one-third of the total water column at the station. A zone of similar thickness was recorded by Levring and Fish (1956), from experiments at the same station in August 1953. Comparison with Figures 3 and 4 shows that the maxima of several algae (*Melosira*, *Cyclotella*, *Nitzschia*, *Pediastrum*) occur at depths where photosynthesis is negligible. Unless heterotrophic nutrition is invoked (as by Ferguson Wood 1956), these maxima must be recruited from cells sinking from the upper productive layers. At the time of the observations practically all the *Melosira* population was in the non-photosynthetic lower layers, and can therefore be assumed to have persisted there in an inactive state since the last period of weak thermal stratification (cf. Fig. 2). This population can be regarded as a reservoir of photosynthetic potential, whose utilization depends upon the extent of vertical mixing determined by thermal stratification.

DISCUSSION

The results of Fish (1957), extended by those described here, show that in its stratification Lake Victoria occupies a peculiarly interesting position among the large tropical lakes so far investigated. For all these lakes small temperature differences have disproportionately large effects upon the distribution of other entities, due to the rapid change in the density of water with temperature at the high temperatures of the tropics. Thus the density change between

24 and 26°C, a temperature range found in Lake Victoria (Fig. 1), equals that between the wider limits of 6 and 12.6°C frequent in temperate lakes. In the deeper African lakes, such as Tanganyika, Kivu, and Nyasa, the resulting density stratification appears to be either permanent or possibly broken in some cases in exceptional years (cf. Beauchamp 1953). By contrast, the stratification of the relatively shallow Lake Victoria (maximum depth about 80 m, mean depth about 40 m: Halbfass 1922) has broken down annually in the years studied, with striking effects upon the distribution of oxygen and phytoplankton. This relative mobility of the stratification is accentuated by the frequent presence of internal waves of large amplitude and long period, which may cause a state of true thermal stratification to be temporarily obscured.

Further complexities arise from the big differences (discussed by Worthington 1930 and Fish 1957) between conditions in the open lake and in the numerous shallow bays and inlets. The bays typically support much denser populations of phytoplankton, and the resulting photosynthetic activity can cause pronounced diurnal changes in the stratification of pH (Worthington 1930, for the Kavirondo Gulf) and oxygen (Talling 1957c, for Pilkington Bay). The thermal stratification here is also more strongly affected by diurnal changes than is that of the open lake, and its breakdown during nocturnal mixing is usually complete. Diurnal changes of stratification in the open lake are described by Worthington (1930), who showed that associated mixing extended to a depth of about 30 m.

The stratification of phytoplankton in the lake still remains very incompletely known, especially as regards seasonal changes. Fish (1957) has shown that such changes are particularly important for the *Melosira* population. Although the present results are insufficient to deduce seasonal changes, they illustrate the effects of a change from a relatively strong to a relatively weak thermal stratification. The resulting trend towards a more uniform vertical distribution of phytoplankton is one familiar from other limnological work. However Figures 4 and 5 show that decided deviations from uniformity still exist with a number of species. The dissimilar patterns of distribution suggest that each species should be considered separately, and that a comprehensive picture of the distribution of phytoplankton within the lake would be far from simple.

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Species Composition of Limnetic Zooplankton Communities[1]

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ABSTRACT

The species composition of limnetic zooplankton communities in 27 Colorado lakes has been determined on the basis of 148 vertical series of plankton-trap samples. Each such series consisted of three or more evenly spaced samples taken from surface to bottom in the limnetic area. Comparable quantitative data for 42 selected lakes in other parts of the world are also considered, such samples having been taken with the plankton trap, pump, Clarke-Bumpus sampler, closing net, etc.

Momentarily, the great majority of Colorado limnetic communities contained one or two species of copepods, one to three species of cladocerans, and three to seven species of rotifers. Comparable data for other small to medium-sized lakes of the world were one to three copepods, two to four cladocerans, and three to seven rotifers. From a species standpoint, therefore, these limnetic communities are remarkably simple. Depending on the complexities of cyclic and seasonal abundance, however, the individual species making up these communities change from one time of the year to another. The most common and widely distributed North American species are contained in the following genera: *Cyclops, Mesocyclops, Diaptomus* (Copepoda); *Daphnia, Bosmina, Diaphanosoma, Ceriodaphnia, Chydorus* (Cladocera); *Keratella, Polyarthra, Filinia, Kellicottia, Conochilus, Asplanchna, Synchaeta* (Rotatoria).

It is unusual to find more than one species in the same genus in a limnetic community at the same time, at least when ordinary quantitative sampling methods are used. But when two species in the same genus do occur together, one is usually 20 or more times as abundant as the other. These and other phenomena are discussed with particular reference to the "niche" concept.

In spite of the cyclic waxing and waning of populations of individual zooplankton species, the great majority of limnetic communities are characterized at any one time by one numerically dominant species of copepod, one dominant cladoceran, and one dominant rotifer. Thus, adequate sampling in "typical" lakes shows that, on the average, 80 per cent of all limnetic copepods belong to one species, 78 per cent of all cladocerans belong to one species, and 64 per cent of all rotifers to one species.

INTRODUCTION

Limnological literature contains hundreds of papers on numerous phases of zooplankton biology, ranging from trivial contributions to the most careful quantitative and qualitative work extending over many months. Because of the wide variety of approaches, emphasis, and methodology, however, it is difficult to review and summarize much of the information contained in these papers. On the other hand, two rather obvious aspects which appear to have been neglected are (1) the structure of limnetic zooplankton communities from the standpoint of the number of species occurring in a population sampled at any one time by means of the usual quantitative methods, and (2) the numerical relationships and relative abundance of these species. Such basic summarized information should be especially pertinent in view of the fact that many biologists have an erroneous idea of the complexity of limnetic zooplankton communities.

The present paper is an attempt to clarify and summarize these two phases of zooplankton biology. A large body of suitable data is available for 27 Colorado lakes, and similar, comparable data from the published literature are available for 42 other selected lakes, mostly outside North America. Only *limnetic* (or pelagic)

[1] Contribution No. 28, Limnology Laboratory, Department of Biology, University of Colorado. This investigation was made possible by means of grants-in-aid from the Penrose Fund of the American Philosophical Society and from the University of Colorado Council on Research and Creative Work. The author is most grateful for such assistance.

zooplankton populations are being considered, *i.e.*, the community of copepods, cladocerans, and rotifers occurring in the open waters of lakes from surface to bottom. (Limnetic protozoans, Diptera larvae, and fortuitous plankters in other taxa are not included.) Such species associations are relatively simple and are sharply distinct from the more complicated zooplankton communities found in the littoral zone and in other areas sufficiently shallow to support a growth of rooted aquatic plants. Some of the data, however, are taken from lakes that are quite small, the smallest having an area of only 7 hectares. Nevertheless, the open waters of such a lake are considered as limnetic, devoid of the influence of rooted aquatics and the shoreline shallows.

Preliminary drafts of this paper were criticized by Dr. W. T. Edmondson, University of Washington, and Dr. C. C. Davis, Western Reserve University. The author is much obliged to them for their helpful suggestions.

SCOPE AND METHODS

Data were gathered from the 27 Colorado lakes over a period of 14 years. Twenty-two are located in northcentral Colorado; five are in western Colorado. Various aspects of the limnology of most of these lakes are discussed by Pennak (1945, 1946, 1949, 1950, 1955a). They represent an extreme range of lake types. For reference purposes these bodies of water are listed as follows: Allens, Barron, Baseline, Bear, Beasley, Beaver, Big, Boulder, Bowles, Brainard, Carp, Eggleston, Estes, Gaynor, Glacier, Gold, Grand, Hayden's, Hotel Twin, Kossler, Loveland, Manchester, Missouri, Owens, Silver, Summit, and Ward. Data for the other 42 lakes are taken from the papers listed in the legend of Table 3.

Sampling procedures were uniform for all 27 Colorado lakes. A "series" consisted of three or more ten-liter Juday plankton trap samples taken within 15 to 50 minutes at regularly-spaced depth intervals from surface to bottom in the deepest area of the limnetic zone. In one lake 80 meters deep, a series included nine trap samples; in typical lakes of ten meters depth about four to six samples were taken; and in shallow lakes of four meters depth only three samples were necessary. The 148 vertical series in Table 1 thus include more than 700 separate ten-liter samples. Some lakes are represented by only a single series of samples; others by as many as 14, taken throughout all seasons. Because of differential depth distribution and the daily vertical migratory pattern of most limnetic zooplankters, a reliable conception of the species present at any one time and their relative abundance makes representative sampling from top to bottom imperative. Mid-water or surface samples alone are worthless.

The Juday plankton trap has been mildly criticized as a quantitative instrument by Ricker (1938) and others. The present writer, however, has had excellent results with it in the small mostly shallow Colorado lakes when vertical series of samples are taken for population estimates. Many replicate samples (unpublished) have established its reliability when properly used. Any presumed "avoidance" of the trap by larger zooplankters was thought to be obviated, for example, by releasing the brass messenger at an appropriate time so that the trap closed before it had been at a particular sampling depth for more than five or ten seconds.

Identifications and counts were made with a Sedgwick-Rafter chamber, and with few exceptions the entire ten-liter sample was counted, not simply an aliquot. "Rare" species were thus detected which might have been missed if another method had been used. This procedure eliminated most of the counting errors and objections noted by such investigators as Serfling (1949) and Littleford, Newcombe, and Shepherd (1940).

For each series of three to nine trap samples the species found were tabulated, and the absolute and relative numerical abundance of each species were calculated and averaged for the whole series. In this fashion an adequate picture of the composition of the limnetic community was derived for the whole water mass from top to bottom.

In the section which immediately follows it must be borne in mind that the discussion

is concerned only with the *momentary* composition of limnetic zooplankton communities. A year-round species list for such communities is often twice or more as long as a list of species present under usual sampling conditions at any one time. This situation, of course, is largely produced by the fact that individual species are usually cyclic and have very short seasonal periods of abundance, and that populations may wax and wane in a remarkably short period of time (see especially Pennak 1949, 1955a).

SPECIES COMPOSITION IN COLORADO LAKES

As indicated in Table 1, the Colorado lakes are notable for the simplicity of their limnetic zooplankton populations. In 99 out of the 148 vertical series of samples taken from the 27 lakes, for example, only a single species of copepod was found, and in 43 series there were two species of copepods. The six series containing no copepods were taken in summer in montane and alpine lakes. *Cyclops bicuspidatus*, a very widely distributed species, was the only limnetic cyclopoid; the other four species of copepods were all diaptomids, of which *Diaptomus siciloides* was by far the most common. With few exceptions, however, when only one copepod was present, it was *Cyclops bicuspidatus* rather than a diaptomid. No single series ever contained two species of diaptomids, although a few lakes sometimes contained one species of diaptomid at one time of the year and a second species at another time.

The situation with respect to the Cladocera was somewhat more complicated. Although nine different limnetic species were collected, the number present at any one time was typically only one or two, the range being none to five species. *Daphnia longispina* was usually present, often along with *Bosmina longirostris*, sometimes with *Ceriodaphnia pulchella*, and more rarely *Chydorus sphaericus*, *Diaphanosoma leuchtenbergianum*, *Ceriodaphnia reticulata*, *Holopedium gibberum*, and *Polyphemus pediculus*. *Daphnia pulex* was found in only seven series and was associated with *D. longispina* only twice (Pennak 1955b). In 17 series *Bosmira longirostris* was the only cladoceran present, and in 11 series *Daphnia longispina* was the only cladoceran.

It is a familiar fact that limnetic rotifers form a much more heterogeneous aggregation than the entomostracans, and this is borne out by the fact that 33 true pelagic species were found in the 27 Colorado lakes. In two series rotifers were absent, and in two series as many as ten species were identified. In 98 series, however, from three to six species were present, with an average of 4.8 species for all series. Because of extreme variations in the abundance of the various species from lake to lake owing to the great differences in seasonal periodicities (see especially Pennak 1946, 1949, 1955a), it is difficult to single out one or two species which might be termed "characteristic" of limnetic communities, but *Keratella cochlearis*, *K. quadrata*, *Polyarthra vulgaris*, *P. euryptera*, and *Filinia longiseta* were most generally found. Other especially common species were *Asplanchna priodonta*, *Conochilus unicornis*, *Synchaeta pectinata*, and *Filinia terminalis*. In general, these are the species of rotifers most abundantly found in the limnetic zooplankton everywhere. One other species, *Kellicottia longispina*, should be added, although it is not common in Colorado lakes.

The simultaneous occurrence of two species of rotifers in the same genus was much more frequent than were similar instances in the cladocerans and copepods.

TABLE 1. *Species composition of limnetic zooplankton in 27 Colorado lakes*

The 148 vertical series of quantitative plankton trap samples are tabulated according to the number of zooplankton species found in each such series.

	Number of species present at any one time											Mean	Median	Total number of species represented
	0	1	2	3	4	5	6	7	8	9	10			
Copepoda	6	99	43									1.3	1	5
Cladocera	17	45	53	24	8	1						1.6	2	9
Rotatoria	2	4	12	21	34	23	20	16	10	4	2	4.8	5	33

Polyarthra vulgaris and *P. euryptera*, for example, were collected together in 24 series, although the former was almost invariably much more abundant. *Keratella cochlearis* and *K. quadrata* are two additional cosmopolitan species, and they were found together in 50 series with the former usually by far the more numerous. A few other comparable examples involved two species of *Brachionus* and two of *Diurella*.

For the Colorado collections it is thus obvious that, from a species standpoint, the momentary composition of the limnetic zooplankton is remarkably simple—so simple, in fact, that the data do not lend themselves to any of several interesting statistical treatments (Fisher, Corbet, and Williams 1943, Brian 1953, Williams 1947). Medians for Copepoda, Cladocera, and Rotatoria were only 1, 2, and 5 species, respectively.

Since these generalizations are based on samples taken during all months of the year, however, the question of possible seasonal differences in species composition should be considered, and such data are therefore summarized in Table 2. Contrary to the implications contained in some textbooks, the number of species present did not vary markedly from one season to another. Copepods ranged from 1.1 species in summer to only 1.5 species in winter; cladocerans ranged from 1.3 species in spring to 2.1 species in autumn and winter; and rotifers from 3.1 species in winter to 5.1 species in summer. Numbers of *individuals* making up the populations, however, are quite another matter, since the warm season densities are usually much greater than those of winter.

Table 2. *Mean number of species of limnetic zooplankters momentarily present in 27 Colorado lakes according to season of the year*

Based on 148 vertical series of plankton trap samples.

	Dec. to Feb.	Mar. to May	June to Aug.	Sept. to Nov.
Copepoda	1.5	1.3	1.1	1.4
Cladocera	2.1	1.3	1.4	2.1
Rotatoria	3.1	4.8	4.9	5.1

SPECIES COMPOSITION IN OTHER LAKES

Unfortunately there is little similarity in plankton sampling methods from one laboratory to another, and the author had to be especially cautious about selecting plankton data from the literature which could be used for comparative purposes. Although many scores of papers were examined, only a disappointingly small fraction were usable. Many papers contained "species lists" of zooplankters collected from a particular lake over a period of time with no indication of which species were found on a particular date. Some papers, such as the excellent work of Rawson (1956), were not usable because the zooplankters were listed by genus only, and there is usually no way of telling whether a particular vertical tow-netting contained one or two species in the same genus. Other papers were useless because they were based on surface tow-nettings, littoral collections, or because the methods of collecting were not explained.

As finally selected, however, the data in Table 3 are limited to 42 lakes where reliable collecting methods appear to have been used so that an accurate picture of the momentary limnetic species composition could be derived for the top to bottom water mass. Samples were taken with plankton traps, Clarke-Bumpus samplers, vertical townettings, plankton pumps, and closing townets at appropriate depths. For some lakes only one series of samples was available, for others as many as 30. A wide variety of small to medium-sized lakes is included, from many latitudes and countries, but no meromictic, highly saline, or polluted waters are considered.

The data for these lakes show more variable conditions than those obtaining in the Colorado lakes. Table 3, for example, shows a range of none to five species of copepods present momentarily at a limnetic station, with a mean of 2.7 and a median of two species as contrasted with a median of only one species for the Colorado lakes. This difference lies mainly in the fact that the former lakes often contained two cyclopoids instead of one, sometimes two diaptomids, and sometimes an additional species of senecellid, centropagid, or temorid. With few exceptions, however, all the copepods

TABLE 3. *Species composition of limnetic zooplankton in 42 lakes, mostly located outside the United States*

Series of quantitative samples are arranged according to the number of zooplankton species found in each series taken at any one time. Data from Baldi, Tonolli, and Pirocchi 1953; Berg and Nygaard 1929; Berg and Petersen 1956; Cushing 1955; Järnefelt 1936a, 1936b, 1952; Juday 1915; Kurasawa, Kitagawa, and Shiraishi 1952; Lindström 1951; Marsh 1898; Rawson 1953; Raymond 1937; Ruttner 1929, 1937; Scheffer and Robinson 1939; Turnowsky 1949; Wolf 1955; Worthington and Ricardo 1936.

	Number of species present at any one time																			Mean	Median
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Copepoda	6	36	136	81	86	9															2.7	2
Cladocera	12	41	86	163	59	22	1	1	3			1									2.8	3
Rotatoria	1	6	10	13	17	45	30	19	5	5	4	3	1	2			1			1	5.5	5

present at any one time belonged to different genera. Thus, Lake Maggiore (Tonolli 1949) commonly exhibited two diaptomids (*Mixodiaptomus laciniatus* and *Eudiaptomus vulgaris*) and two cyclopoids (*Cyclops strenuus* and *Mesocyclops leuckarti*) at the same time. In general, medium-sized lakes contain more species of copepods momentarily in the limnetic zone than do small lakes, and this perhaps also explains why the relatively small Colorado lakes usually had fewer species than those lakes included in Table 3.

If, however, copepod data for very large lakes are considered, it is obvious that their populations commonly consist of a markedly longer list of species. Rawson (1956), for example, often found the following in a single vertical tow-netting in Great Slave Lake: *Diaptomus* spp. (?) (diaptomid), *Limnocalanus macrurus* (centropagid), *Cyclops* spp. (?) (cyclopoid), *Senecella calanoides* (senecellid), and *Epischura lacustris* (temorid). Although Chandler (1940) and Davis (1954) do not give species lists for single vertical series of samples from Lake Erie, it is obvious from their discussions that they frequently found six or more species of copepods in a series.

Limnetic cladoceran populations are also usually more complicated in lakes other than those studied in Colorado, since the median is three for the former and two species for the latter. Usually, however, (and in contrast to the copepods) the species involved are almost all cosmopolitan and common to both sets of data. It is rather striking that all series of samples containing six, seven, eight, and 11 species (Table 3) were taken in Finnish lakes (Järnefelt 1936a, 1936b). The significance of these large numbers of species is unknown, but perhaps it is a reflection of the thoroughness of the investigation or the methods used.

There is also considerable evidence to show that vertical series of samples taken from the largest lakes commonly contain more species than series taken from small to medium-sized lakes. Thus, the papers of Chandler (1940) and Davis (1954) appear to show that five or more species were often collected together.

An important paper by Borecky (1954) should be noted. She made frequent qualitative collections of limnetic Cladocera from Pymatuning Reservoir, Pennsylvania, over a three-year period but found only four species—*Bosmina longirostris*, *Chydorus sphaericus*, *Daphnia longispina*, and *Diaphanosoma brachyurum*. A later paper (Borecky 1956) based on one year's quantitative collecting in the same lake includes small numbers of a fifth species, *Ceriodaphnia reticulata*.

Rotifer populations gave remarkably similar results for both Colorado and elsewhere, since the median was five species for the two sets of data. In general, the same group of cosmopolitan species and genera is involved. Again, most of the series containing ten or more species were taken by Järnefelt.

Emphasis should be placed on the fact that neither latitude nor altitude appears to have any important correlations with the number of species present in the limnetic plankton. Tropical, temperate, and subarctic lakes are all strikingly similar. Although some of the plankton-trap series

taken from the highest Colorado lakes contained fewer species than usual, there were also low altitude series exhibiting the same situation at certain times of the year. Likewise, no significant differences in species numbers were found between oligotrophic and eutrophic limnetic areas.

NICHE CONCEPT IN LIMNETIC ZOOPLANKTON COMMUNITIES

The idea of a "niche" in the ecological sense has been given different interpretations and shades of meaning in various research papers and textbooks (see especially Allee *et al.* 1949). In the "habitat niche" sense the limnetic water mass is essentially a continuum of ecological factors to which all zooplankters are being exposed. To be sure, there are vertical differences in various ecological factors, but aside from the conditions imposed by summer oxygen exhaustion in the hypolimnion of some lakes, most zooplankters appear to move about remarkably freely through a limnetic water mass, both vertically and horizontally, especially when an entire 24-hour period is taken into consideration. This is in striking contrast to the littoral zone where the bottom and its associated debris and rooted aquatics provide an additional wide variety of substrates, and, therefore, habitat niches. In the present paper, however, "niche" is used in the "food niche" or "functional niche" sense, *i.e.*, the complicated interrelationships that any species has with such factors as food, feeding, predators, prey, etc.

Although it is easy to speak of the functional niche of a particular species in ambiguous, general terms, it is quite another matter to define that niche in terms of the precise ecological requirements of that species. And, in fact, it is generally recognized that such details are poorly known for zooplankters. Commonly, however, and for the sake of convenience, animals are relegated to a niche chiefly on the basis of their food habits with little regard to other requirements.

Among copepods, for example, the cyclopoids have the mouth parts modified for seizing unicellular plants and animals, as well as bits of debris. Fresh-water calanoids, however, are less selective filtration feeders which subsist on all types of living and dead particulate material strained from the water by the efficient mouth parts, especially the maxillae. As inferred in the preceding paragraphs, a limnetic plankton population sampled with the usual field methods at any one time is commonly found to have just one species in each of these functional or "food niches"—a cyclopoid and a calanoid, and especially in the United States these are more often than not one of the following cyclopoids: *Cyclops bicuspidatus*, *C. vernalis*, and *Mesocyclops leuckarti*, and one of the following calanoids: *Diaptomus sicilis*, *D. siciloides*, *D. oregonensis*, and *D. minutus*. If two species of limnetic cyclopoids are found together they are almost invariably of different genera. Similarly, when two limnetic calanoids are found together, it is very seldom that they are both species of *Diaptomus*; rather it is one *Diaptomus* and an additional species of *Limnocalanus*, *Epischura*, or *Senecella*, particularly in large, cold lakes of North America. Similar associations of genera occur in other parts of the world. In Europe, for example, when limnetic populations are found to contain two calanoids, these are often one species of *Mixodiaptomus* and one species of *Eudiaptomus*. The extensive work of Carl (1940) on Canadian lakes is additional strong evidence for the fact that a genus of copepods or cladocerans is seldom represented by more than one species in limnetic samples.

It is assumed that the niche requirements in different limnetic genera of copepods are sufficiently distinct to permit the coexistence of populations of a species in each of the genera, and it is sometimes possible to distinguish primary differences in the ecological demands of such genera. On some few occasions, however, limnetic samples from small and medium-sized lakes contain two species in the same genus, a situation which is difficult to reconcile with the familiar hypothesis of Gause, since they are presumed to be in effective ecological competition for the same niche or for two niches which are almost identical. But a review of the literature shows that under such cir-

cumstances one species is numerically dominant and usually 20 or more times as abundant as the other species. A few examples of the uncommon simultaneous occurrence of two species of limnetic copepods in the same genus as determined by ordinary sampling methods are: *Diaptomus oregonensis* and *D. minutus* (Langford 1938, Tressler and Bere 1936), *D. denticornis* and *D. laticeps* (Lindström 1951), *D. sicilis* and *D. minutus* (Marsh 1898), *D. sicilis* and *D. nevadensis* (Comita 1953), *Eucyclops agilis* and *E. prasinus* (Juday 1915), *Cyclops vernalis* and *C. bicuspidatus* (Andrews 1953). One possible explanation of such associations has been suggested by Hutchinson (1951), who notes that a size difference between two species of copepods in the same genus may very well account for quite different selectivity of food and therefore less interspecific competition than might be inferred. A concrete example of this phenomenon has been reported for two closely related genera by Fryer (1954) who found in Lake Windermere that the digestive tract of *Arctodiaptomus laticeps* (1.54 to 1.65 mm long) contained *Melosira*, but that *Eudiaptomus gracilis* (1.14 to 1.23 mm long) fed on minute algae and tripton.

In spite of great morphological and life history differences from one genus of limnetic cladocerans to another, very little is known about significant differences in their ecological requirements and niches. To be sure, there are some well known cases of different generic and species preferences in temperature, alkalinity, and water chemistry, but to all outward appearances most species seem to have the same generalized grazing habits and mechanisms by which they filter out whatever microscopic living and dead particulate organic materials may be present and readily available in the water (a few exceptions, such as *Leptodora*, are predaceous). And from the food standpoint, it appears that most species of limnetic cladocerans compete for essentially the same common pool of materials.

Nevertheless, as emphasized in the foregoing section, a limnetic population, according to ordinary sampling methods, usually consists of two to four species, each species being in a different genus. The most common exception is the co-occurrence of *Daphnia pulex* and *D. longispina*, sometimes at different depth strata. In general, there seems to be little to distinguish between the ecological requirements of these two species. *D. pulex* is, of course, the larger form; it is sometimes said to be much less characteristic of large, open waters than is *D. longispina*, but there are many exceptions. Both species are occasionally reported from the same lake, but usually their periodicities are quite different so that their simultaneous occurrence in large numbers is uncommon. Woltereck (1932) reported *D. pulex*, *D. longispina*, and *D. longiremis* together in several northern Wisconsin lakes, but at least during the daytime all three species had their greatest densities at distinctive and different depths. Similar differential depth stratification for two species of *Daphnia* is reported from two Japanese lakes by Uéno (1939). It should be mentioned that the North American species of *Daphnia* are being revised, and it is very likely that this revision will show that *D. pulex*, for example, is actually a complex of species. Our concept of their roles in the limnetic plankton may accordingly require considerable modification. Aside from this *Daphnia* problem, only a few papers can be found which record the co-occurrence of two or more species of limnetic cladocerans in the same genus. Worthington and Ricardo (1936), for example, found three species of *Ceriodaphnia* together in Lake Edward.

A few limnetic rotifers, such as *Asplanchna* and *Synchaeta*, are at least partly predaceous, but most of the characteristic genera (*Keratella*, *Polyarthra*, *Filinia*, *Kellicottia*, and *Conochilus*) feed upon small particulate materials, both living and dead, by concentrating them at the mouth through currents generated by the coronal cilia. From the standpoint of their obvious food and feeding habits, they occupy essentially the same niche, and although much generalized ecological work has been done on the rotifers, it has not been sufficiently critical to distinguish the differences in the total functional niche pictures of these genera and species.

In reviewing the suitable literature on

limnetic rotifer populations, it appears that the situation is generally similar to that found in Colorado. That is, a community consists of a small number of cosmopolitan species, and each such species is ordinarily the only one representing its genus. The usual exceptions involve finding *Keratella cochlearis* and *K. quadrata* together, two species of *Polyarthra*, and more rarely two of *Synchaeta*, two of *Ploesoma*, two of *Conochilus*, or two of *Brachionus*. Whenever two species in the same genus are present, one is almost always much more abundant than the other. At this point it should be emphasized that a limnetic species list for the whole year usually reveals more than one species in several rotifer genera, but the cycles of abundance for such pairs of species are short, unpredictable, and seldom in phase.

The foregoing paragraphs give considerable support to some of the principles stated by Elton (1946) as clarified by Bagenal (1951), and especially to Elton's contention that a high percentage (86%) of the genera present (momentarily) in an animal community (as determined by adequate sampling) are represented by only a single species. The relative simplicity of the limnetic community with respect to species, generic composition, and functional niches recalls Elton's closing sentence: "The ability of certain groups of species mostly separated by generic characters, to exist together on the same area while drawing upon a common pool of resources, is one of the central unsolved problems of animal community structure and population dynamics." From the standpoint of the zooplankton, Riley (1953) presents some interesting conjectures on this matter.

The discussion thus far has been based on "adequate" momentary sampling in the limnetic zone, and very properly we might ask ourselves how the species picture would change if mass sampling could be carried out. In other words, if 100 limnetic vertical tows or 100 vertical series of plankton trap samples could be taken from a lake within a day's time, how many species of copepods, cladocerans, and rotifers would these samples contain? The answer to such a hypothetical question is, of course, highly conjectural, but it is probable that perhaps double or triple the number of species would be found as compared with the number present in a single vertical tow or a single vertical series. These additional species would undoubtedly be in the "rare" category. Nevertheless, such rare individuals are essential as "seed" nuclei for population cycles to develop at later dates, in addition to or in the place of other species. The role of such rare or seed individuals is probably aided by the occurrence of winter eggs, resting eggs, or encystment stages in the surface mud of the bottom deposits in the limnetic zone. There is also a strong possibility that the littoral zone contains a small and continuing reservoir population of limnetic species. In fact, none of the species mentioned in this paper is necessarily *restricted* to limnetic areas.

SPECIES DOMINANCE

Any aquatic biologist who has spent much time counting quantitative limnetic zooplankton samples has been impressed with the fact that a sample usually contains one species of copepod, one cladoceran, and one rotifer which are exceptionally abundant and clearly dominant numerically over the other species in those groups. In spite of the rather obvious nature of this phenomenon, it does not appear to have been emphasized, nor has any summary account of it been published.

Such data are therefore summarized in Table 4 for the 27 Colorado lakes and the 148 vertical series of limnetic plankton samples; comparable data are also shown for 22 other selected lakes located outside of Colorado. The latter are all included in the references listed in the legend of Table 3.

It is at once apparent from this table that species dominance is a valid and well-defined generalization. Only rarely does the most abundant species of copepod, cladoceran, or rotifer account for less than 50 per cent of all of the copepods, cladocerans, or rotifers present in a vertical townetting, vertical series of trap samples, etc. To a certain extent this principle is a reflection of the fact that only a few species are involved in a typical population, but perhaps of greater fundamental importance is the

TABLE 4. *Percentage composition of momentary limnetic zooplankton populations for 27 Colorado lakes and 22 lakes from other parts of the world*

	Zooplankton	Most abundant species		Second most abundant species		All other species present	
		Range	Mean	Range	Mean	Range	Mean
27 Colorado lakes	Copepoda	50.4–100.0	92.9	0.0–49.6	7.1	—	—
	Cladocera	39.7–100.0	84.7	0.0–49.2	12.8	0.0–28.8	2.5
	Rotatoria	30.3–100.0	63.6	0.0–48.9	21.4	0.0–42.3	15.0
22 extra-Colorado lakes	Copepoda		78.8		17.6		3.6
	Cladocera		72.9		21.3		5.8
	Rotatoria		64.4		18.7		16.9

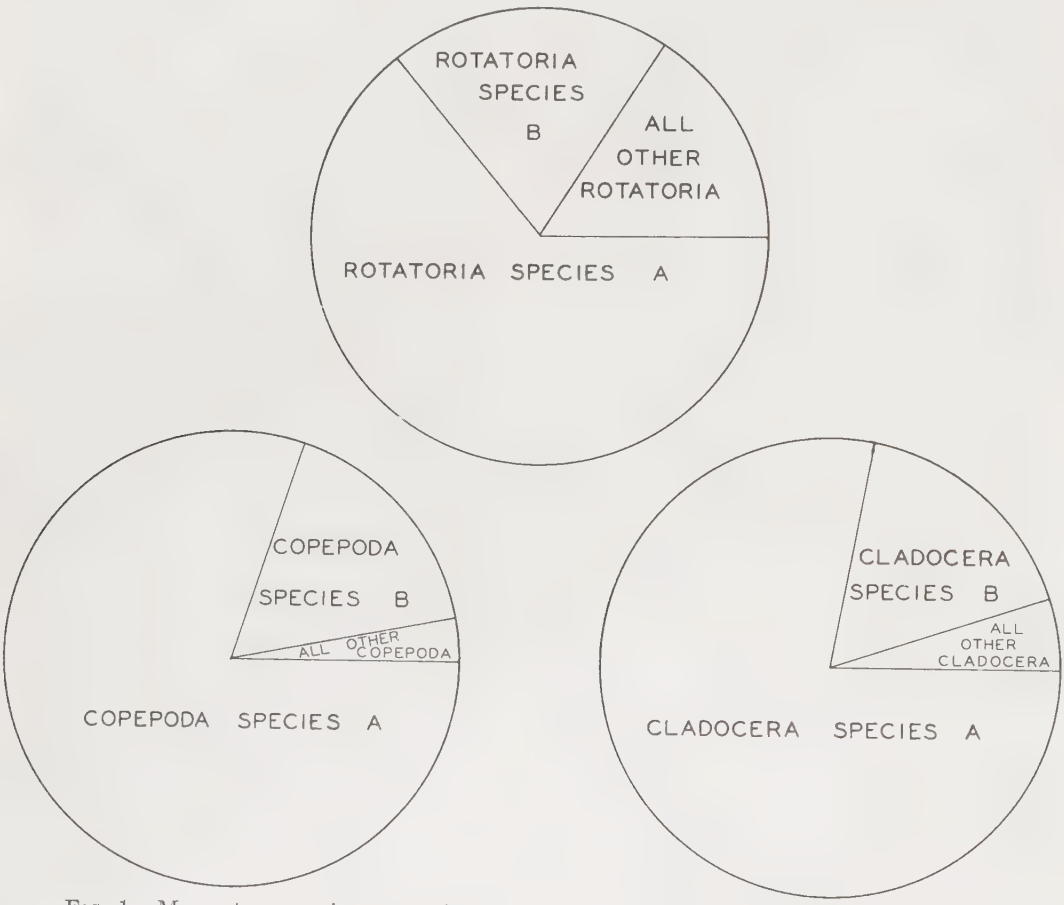

FIG. 1. Momentary species composition of a typical limnetic zooplankton community.

fact that one species in each of the three zooplankton categories presumably has certain genetic, morphological, physiological, or ecological features which momentarily enable it to utilize its habitat more efficiently and to greater advantage. On the other hand, it should be borne in mind that a particular species attains numerical dominance only in a transient sense, since it characteristically is cyclic in its seasonal occurrence and may be abundant at one time and represented by only a few individuals a short time previously or subsequently (see Pennak 1946, 1949, 1955a). Thus, if the limnetic zone of a particular lake is sampled at biweekly intervals during

the year, it may be that as many as four or five different species of rotifers exhibit numerical dominance at various seasons.

Table 4 shows a considerable difference between the copepod data for Colorado lakes and those for other lakes. This is chiefly due to the fact that the former contained only one or two species of copepods at a time in contrast to one to four species in the latter. As representative of lakes the world over, however, the results for the extra-Colorado lakes are undoubtedly more nearly "typical." That is, the most abundant species of copepod at any one time accounts for about 80 per cent of all copepod individuals present, the second most abundant species accounts for about 17 per cent of all copepods, with the remaining 3 per cent containing the third, fourth, etc. species.

There are also some differences between the cladoceran data for the Colorado lakes and the extra-Colorado lakes, but they are less pronounced, and an "average" picture is probably somewhere between the two sets of data. In a typical limnetic situation, then, the most abundant cladoceran at any one time accounts for about 78 per cent of all individuals, a second species accounts for about 17 per cent of all individuals, and additional species amount to the remaining 5 per cent.

The two sets of data for rotifers are remarkably similar, with the most abundant rotifer accounting for 64 per cent of all individuals, a second species accounting for 20 per cent, and all other species (sometimes as many as six or more) totaling 16 per cent.

A summarized concept of the "average" species composition of a limnetic zooplankton community as determined by the usually reliable quantitative field methods is shown in Figure 1. Note especially the similarity of the Copepoda and Cladocera portions of this figure. In considering such a diagram, it should be borne in mind that the relative sizes of the sectors in each circle are indicative only of *percentages* and not absolute numerical relationships, but that these percentages are valid for both dense and scanty zooplankton populations.

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Preliminary Observations on Cobalt and Vitamin B_{12} in Fresh Water[1]

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ABSTRACT

The cobalt, copper, and iron contents of a vertical series of water samples from Linsley Pond are given. Cobalt and iron are higher in the hypolimnion than in the epilimnion, but copper is not. The cobalt and iron content of the seston is estimated to be about equal to the content in normal soils, but the copper content is much higher. Vitamin B_{12} only exists in the water at concentrations lower than the optimum for some algae in culture. The proportion of the total cobalt tied up in vitamin B_{12} in the epilimnion is about 10% and about 4% or less in the hypolimnion.

INTRODUCTION

The biological significance of cobalt

Cobaltous ion is known to act as an alternative cofactor for magnesium or manganese in a number of enzyme systems (McElroy and Nason 1954), but there has never been any conclusive evidence that the element is required by any organism except as a component of the structure of vitamin B_{12}. The need for vitamin B_{12} is widespread in nature and one specialist in the field (Marston 1956) has recently suggested that all animals require the vitamin. A number of microorganisms including bacteria, fungi, and algae also require vitamin B_{12}. Higher plants do not synthesize the vitamin; its synthesis seems restricted to some bacteria, fungi, (not including yeasts), blue-green algae, and perhaps some red and brown marine algae. The fact that the synthesis of the vitamin is thus restricted has important ecological consequences for animals. The very widespread malady of cattle and sheep variously called pining, salt sickness, bush sickness, coast disease, etc., was shown in 1935 to be a cobalt deficiency (Marston 1935). It was later demonstrated that vitamin B_{12} was the entity involved (Marston and Smith 1952) and that cobalt in the fodder is only important to the extent that the microflora of the rumen requires cobalt to maintain vitamin B_{12} synthesis at a level high enough for its own proliferation and incidentally for the needs of the host.

The extent to which cobalt or the vitamin B_{12} supply limits animal growth and distribution in the various types of communities is an important question. In the aquatic realm it has often been observed that manure in water causes the prolific growth of algae. The mineral content of such fertilizer is undoubtedly of some importance, but the fact that vitamin B_{12} may be involved should also be considered. Euglenids and chrysomonads, which require vitamin B_{12} but often no other organic moiety, are often found blooming in pasture pools (Hutner *et al.* 1950). The extent to which vitamin B_{12} may limit the distribution of these groups should not be underestimated. It is the purpose of this paper to present some data on cobalt and vitamin B_{12} in fresh water in an attempt to clarify the ecological picture.

The geochemistry of cobalt

The cobalt content of the earth's crust has been estimated at about 20 parts per million (Rankama and Sahama 1950); basic rocks and oxidate sediments are high in cobalt; silicic rocks, hydrolysate sediments, and especially limestone are low in cobalt. Normal soils contain about five to ten parts per million, but soils with less than one part per million are not uncommon. What is of

[1] Based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Yale University 1956. The writer would like to express his appreciation to Prof. G. E. Hutchinson, Dr. E. S. Deevey, and Dr. V. T. Bowen for their inspiration and assistance.

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greater importance ecologically in soils is the availability of cobalt and this may bear no relationship to the cobalt content as ordinarily determined (Wright and Lawton 1954).

Cobalt in fresh water

Cobalt has been determined quantitatively in fresh water by only a few workers. A summary of the data of the various authors is given in Table 1 in which nickel data are given for comparison.

It is readily apparent from Table 1 that cobalt exists in water in the range of parts per billion. It should also be noted that more often than not cobalt was not detected in the dried residues of fresh waters. The sensitivity of the simple spectrographic techniques for cobalt is about 1 part in 100,000, so it is very likely that the samples in which cobalt was not detected contained less than 1.0 ppb, and perhaps not a tenth of this amount. The values of Maliuga are higher than all but the highest values of Lopez de Azcona who indicated that two of his samples in which cobalt was detected were "ferruginous." Maliuga used polarographic analyses of rubeanic acid precipitates from water samples of less than ten liters. The writer's experience with the polarograph has been that it is not possible to determine the quantity of cobalt in 10 L of ordinary surface water by polarographic means.

TABLE 1. *The cobalt and nickel contents of fresh waters according to various authors*

Author, location	Cobalt ppb	Nickel ppb
Braidech & Emery (1935)		
U. S. tapwaters	traces in only 3 of 24 dry residues from samples of 2 L	1.0–10 in 13 samples; 3 other samples had 20, 40, and 300
Strock & Drexler (1941)		
Saratoga, N. Y. mineral water	0.11	0.27
Maliuga (1946)		
U. S. S. R.		
Rivers of W. Russia	1.9–6.7	1.2–13.0
Mountain streams	0.74–19.0	1.3–75.0
Freshwater lakes	2.3–6.5	5.0–19.0
Lopez de Azcona (1947)		
Iberian peninsula "medicinal" waters	detected in 18 of 138 residues 0.02–20*	detected in 29 residues 0.2–200*
Mitchell (1952)		
South Carolina creek water	0.26	—
Wilska (1952)		
Finland ground waters	detected in only 2 of 71 dry residues 0.02, 0.023*	detected in 5 residues mean 0.07*

* These figures were originally given in terms of the dried residues only; the solids content of the waters was not given. The writer has converted them to a wet basis by assuming that the samples had a total solids content of 200 ppm, which seems reasonable for average river water based on Clarke's (1924) review.

Vitamin B_{12} in fresh water

Apparently the only report of the determination of vitamin B_{12} in fresh water is that of Robbins *et al.* (1950, 1951) who assayed the water of a small pond at the New York Botanical Garden that periodically developed blooms of *Euglena*. Robbins *et al.* using *E. gracilis* for the assay found from 0.1 to 2.0 micrograms vitamin B_{12} per liter throughout the year with an average of about 0.5. The bottom material was not assayed but an extract of the soil from around the pond was found to contain the equivalent of 2 to 15 micrograms per kilogram of fresh soil.

MATERIALS AND METHODS

During the years 1951 and 1952 the writer collected large samples of lake and creek water in Connecticut and analyzed the samples for cobalt, copper, and iron. Attention was largely restricted to Linsley Pond, a small glacial lake in North Branford, Conn. Linsley Pond has been the subject of a considerable body of limnological research (Riley 1939, 1940; Hutchinson 1941). Linsley Pond lies close to the eastern edge of the central lowland of Connecticut. The most prominent feature of the local geology is a Triassic red and gray sandstone and conglomerate cut by intrusive sills and dikes of traprock. Much of the area is covered by glacial debris of diverse nature and origin. The drainage basin of Linsley Pond is not regionally atypical. It is not likely that the basin is cobalt-poor.

Water samples were collected in a weighted bottle sampler similar to the Meyer sampler shown on p. 23 in Ruttner

(1953). The rubber stopper of the wide-mouthed bottle was fitted with a steel eyelet and a bent sealed glass tube. In operation, the sampler was lowered to the desired depth by a metered lowering line. A second line attached to the stopper eyelet and looped around the glass tube was played out slack. When the bottle had reached the desired depth, the slack line was jerked abruptly breaking the glass tube and allowing water to run into the bottle. After a few minutes the stopper could be pulled out with a second jerk on the slack line and the bottle would then fill in a few seconds.

The samples were evaporated on a water bath under vacuum. The water was filtered through a medium fritted glass filter as it was drawn up out of the sample bottle into the boiler flask of the evaporator. The material which settled in the sample bottle or was collected on the filter has been designated seston. The residue from the evaporation of the filtered water has been designated the soluble fraction. The distinction between the fractions might unfortunately be only an artificial one in the present work for the following reasons. In preliminary determinations it was found that samples that were stored at room temperature for more than a few days contained no cobalt in the soluble fraction with but few exceptions. When five micrograms of cobaltous ion was added to a nineteen-liter sample of lake water and the sample evaporated, half of the added cobalt was recovered in the seston and only half in the soluble fraction. The evaporation of a nineteen-liter sample took about twenty-four hours even under the best conditions. In the ultimate series of analyses samples were only collected as they could be processed, and when it was necessary to store a sample for a day or two this was done at 4°C.

Evaporation residues (the soluble fraction) and seston were analyzed separately in any case. The residue was washed out of the boiler flask with a minimum of nitric acid, digested in a casserole to dryness, and taken up in a little hydrochloric acid. The pH was adjusted to 2.0 with ammonium citrate and ammonium hydroxide.

The solution was then extracted with successive small portions of dithizone in carbon tetrachloride. The carbon tetrachloride portions were combined and conserved for evaporation, wet ashing, and the colorimetric determination of copper by the dithiocarbamate method (Sandell 1950). The pH of the aqueous phase was then adjusted to 8.5 with ammonium hydroxide and extracted again with dithizone in carbon tetrachloride. The carbon tetrachloride portions were combined for evaporation, wet ashing, and the colorimetric determination of cobalt by the nitroso-R-salt method (Sandell 1950). Iron was determined on small raw samples by the orthophenanthroline method (Sandell 1950).

Seston was washed out of the filter and sample bottle with nitric acid and digested in a casserole. The wet ashing was completed with a little perchloric acid. The citrated solution was then extracted with dithizone solution exactly as for the water residues, and copper and cobalt were determined on the extracts.

Vitamin B_{12} was assayed in raw water samples using *Euglena gracilis* and the tube method described by Robbins *et al.* (1950). The assays were performed by Mrs. P. R. Burkholder whose assistance is gratefully acknowledged.

RESULTS

A number of lake water samples were evaporated and the residues analyzed while the methods were being developed. Several of these samples gave blank results. A 12-liter sample of distilled water was evaporated and analyzed and gave blank results. Ten micrograms of cobaltous ion added to a 12-liter sample of distilled water were recovered quantitatively in the analysis. Table 2 contains the results of the cobalt analyses on preliminary samples; all of the preliminary samples had been stored at room temperature for several days or longer before being evaporated. It is well known that lake water samples stored at room temperature soon develop a coating of bacteria on the bottom and sides of the container. This material and any material absorbed from solution by it would be included in the seston fraction because of the filtering technique.

Some of the samples in Table 2 deserve brief comment. The sample from Schoolhouse Cove in Lake Zoar was evaporated before the evaporator was equipped with the fritted glass filter; the device used for this sample gave very poor filtration. The sample from Berkshire Brook although very high in humic color, had so little seston that no analysis of that fraction was made.

During August of 1952 water samples for cobalt and copper analyses were collected from six depths in Linsley Pond. Temperature and dissolved oxygen data for 15 August 1952 revealed that the lake was stratified, with the thermocline between 5 and 6 meters and less than 10% oxygen saturation below 8 meters. Samples from 8 meters and below had a strong odor of hydrogen sulfide when collected. Table 3 contains the oxygen and temperature data for the water column of 15 August 1952; Table 4 shows the cobalt, copper, and iron content for samples collected at various times in August 1952.

Samples of Linsley Pond water for vitamin B_{12} assay were collected on 14 October 1952 (Table 5). Temperature measurements on that date indicated that stratification of the bottom water had persisted, even though the epilimnion was colder by about 10°C than on 15 August.

TABLE 2. *Miscellaneous cobalt determinations on fresh waters of Connecticut*

Sample	Depth m	Date	Cobalt ppb	
			Soluble	Seston
Lake Zoar: School house Cove	0	5 June 1951	0.11	nf
Lake Zoar	15	4 Aug. 51	nf	0.14
Candlewood Lake	0	5 June 51	nf	nf
Berkshire Brook "Bog water"	0	13 Dec. 51	0.05	—
Linsley Pond	0	14 Aug. 51	nf	nf
	2	14 Aug. 51	nf	0.08
	11	14 Aug. 51	nf	0.16
	14	14 Aug. 51	0.08	0.05
	12	Apr. 52	nf	0.05
	0	May 52	nf	0.21

nf indicates analyzed for but not found; these samples contain less than 0.02 ppb.

TABLE 3. *Temperature and dissolved oxygen in Linsley Pond, 15 August 1952*

Depth m	Dissolved oxygen mg/L	Temperature °C	Saturation value mg/L	Per cent saturation
0	6.81	27.2	8.1	84
3	6.58	25.0	8.4	78
5	4.26	13.3	10.5	41
7	1.87	8.3	11.8	16
8*	1.30	7.6	12.0	11
9	0.52	7.2	12.1	4.3
10	0.55	7.0	12.2	4.5
11	0.50	6.9	12.2	4.1
12	0.39	6.8	12.3	3.2

* Odor of H_2S in samples below 8 meters.

DISCUSSION

While admittedly it would have been ideal to be able to analyze a vertical series of samples collected on the same day, the evaporation of such large samples as were employed took so long that it would have been necessary to store some of the samples longer than was deemed prudent. During the week in August 1952 in which the ultimate series was collected there were no storms, and since Linsley Pond is sheltered it is very unlikely that any vertical mixing occurred.

The reader might well wish that analyses

TABLE 4. *Cobalt, copper, and iron from a vertical series of water samples, Linsley Pond, August 1952*

Depth m	Date	Cobalt ppb			Copper ppb			Iron ppm	
		Soluble	Seston	Total	Soluble	Seston	Total	Soluble	Total
0	19 Aug.	nf	0.042	0.042	2.48	1.42	3.90	0.00	0.62
2	27 Aug.	nf	0.021	0.021	2.00	1.06	3.06	0.00	0.26
3.5	27 Aug.	nf	0.025	0.025	1.63	0.42	2.05	0.00	0.20
5	19 Aug.	0.025	0.042	0.067	5.10	1.37	6.47	0.12	0.40
8	22 Aug.	0.042	0.063	0.105	1.21	0.68	1.89	0.84	1.08
12.5	22 Aug.	0.021	0.032	0.053	2.00	0.68	2.68	4.40	—

nf indicates not found in the analysis; these samples would contain less than 0.020 ppb.

TABLE 5. *Vitamin B_{12} in Linsley Pond samples by* Euglena *assay*

Depth in meters	0	2	3.5	5	8	16
Vitamin B_{12} μg/L	0.075	0.075	0.060	0.060	0.065	0.150

had been run in duplicate and indeed so does the writer, but the processing of each sample took so long a time by the method employed that it was not possible to perform more analyses with the time and facilities available. The writer's confidence in the reliability of the analyses in the absence of any full-scale duplicate analyses is necessarily somewhat subjective, but it is based on the repeated recovery in the analyses of microgram quantities of added cobalt, and on the fact that there were many samples analyzed which gave blank results. In no case was there any evidence of contamination from reagents, glassware, etc. As will be seen in the subsequent discussion, there is nothing apparent in the aggregate data and their consequences that casts serious doubt on their reliability.

One of the most striking things about the vertical distribution of cobalt in Linsley Pond during stratification is the obvious enrichment in the hypolimnion especially with regard to soluble cobalt. The same is true of iron but not at all of copper, which decreases markedly below 8 meters. The solubility of copper sulfide at various pH levels can be calculated according to the theoretical treatment of Hutchinson (in press). The calculation indicates that ionic copper would not exist at a detectable level of concentration in the presence of the amount of sulfide ion in equilibrium with an excess of ferrous sulfide such as exists in the Linsley Pond bottom waters. The presence of free H_2S in the system would further limit the ionization of copper sulfide. The small quantity of copper that was detected at 8 and 12 meters unquestionably represents copper in soluble complexes or as colloidal or suspended particulate matter (probably copper sulfide) fine enough to pass the filter.

The maximum concentration of cobalt that can exist in the presence of FeS is 8.7×10^{-4} mg/L per mg iron. At 8 and 12 meters in Linsley Pond only 4.2×10^{-5} and 2.1×10^{-5} mg of soluble cobalt per liter were present, and these are equal to 5.0×10^{-5} and 4.8×10^{-6} mg/L/mg Fe. The concentration of sulfide ion present at these depths was undoubtedly higher than the iron content would suggest since free H_2S was so obviously present. It may also be true that the quantity of cobalt present represents all that the sediment can deliver to the hypolimnion between the spring circulation and August.

The average total content of cobalt, copper, and iron for Linsley Pond is: cobalt 9.1×10^{-5} ppm, copper 3.4×10^{-3} ppm, and iron 1.2 ppm. The cobalt content is of the same order of magnitude as only the lowest figures in Table 1, but it should be remembered that many investigators failed to detect cobalt at all in the bulk of their samples. The quantitative data in the literature may represent only waters high in cobalt.

The cobalt, iron, and copper content of Linsley Pond seston

Riley (1940) gave the average seston content of Linsley Pond water as 1.94 mg dry seston per liter. This average involved samples filtered throughout the year with a 35-second membrane filter, which undoubtedly gave more efficient filtration than the fritted glass used in the present work. Riley estimated that only 17–28% of this seston was phytoplankton. The mean seston cobalt content of Linsley water in the present work was 3.75×10^{-5} mg/L. Combining this cobalt content with Riley's seston value for the water, gives 19 mg/kg for the cobalt content of dry seston. This cobalt content (19 ppm) is of the same order of magnitude as some soils, but it is higher than most, and it is about twice the concentration found by the writer in Linsley Pond surface sediment (8 and 11 ppm). It is one or even two orders of magnitude higher than the cobalt content of most plant and animal tissues (Bertrand and Mokragnatz 1925a, 1925b). Of course seston contains not only phyto- and zooplankters and their remains but also variable quantities of mineral silt and colloidal material.

The six curves for the vertical distribution of copper in Linsley Pond given by Riley

(1939) yield an average for seston copper of 0.010 mg per liter of water. Combining this figure with Riley's estimate for the seston content of the water (1.94 mg/L) leads to an estimate of 5,160 ppm for the copper content of dry seston. In the present work the mean seston copper was lower (0.00087 mg/L), which leads to an estimate of 485 ppm for the copper content of dry seston. The copper content of normal soils is about 20 ppm according to Rankama and Sahama (1950). Riley (1939) found as high as 349 ppm in the leaves of oak, elm, and maple. The writer found 444 ppm in the surface sediment of Bantam Lake in Connecticut, and Nichols *et al.* (1946) reported that as high as 1000 ppm can be found in the surface muds of lakes that have been treated with copper sulfate for algae bloom control. Both Bantam Lake and Linsley Pond have been irregularly and ineffectively treated, but Linsley Pond had not been treated before 1940. While the copper content given for Linsley Pond seston is admittedly only an estimate, it does suggest that the suspended material in lake water, from which the lake sediments are derived, can have a very high copper content even in the absence of copper treatment.

The iron content of Linsley seston can be calculated from the data of Hutchinson (1941) and unpublished data from the field notebooks for that study. Based on the iron content of Linsley water, the dry seston content, and the seston ash content of the water, dry seston from Linsley Pond has an iron content of 3.6%, which is comparable to a 3.5% estimate for soils given by Maliuga (1946). Seston is markedly more organic than soils, and the comparison between them on a dry weight basis is esentially unjust. On an ash basis seston contains about three times as much cobalt (63 ppm) and iron (12%) as soils, and vastly more copper. If seston is in reality so very rich in copper, then filter-feeders such as cladocerans get a diet which would be considered toxic in almost any sense. This tends to confirm the idea that filter-feeding is a selective process—that filter-feeders are capable of separating algal and bacterial cells from detritus and colloid particles, which contain the bulk of the copper. Poulson *et al.* (1952) showed that copper bound to yeast cells is not absorbed from the food medium by the midgut of *Drosophila* larvae as "soluble" copper is. It is thus entirely possible that a process of selective absorption rather than selective filtration is involved in the alimentation of zooplankton; the particulate copper may not be absorbed even though it passes into the gut.

The efficiency of synthesis of vitamin B_{12} in Linsley Pond

It has been mentioned in the introduction that the efficiency of vitamin B_{12} synthesis may be very important in some ecological systems. If the total cobalt concentrations in the various levels of Linsley Pond are compared with the cobalt equivalent of the vitamin B_{12} found at the same levels, it is apparent that between two and thirteen per cent of the cobalt is tied up in the vitamin (Table 6). This conclusion is based on the assumption that the August and October columns of water are comparable; the persistence of stratification would suggest that they are. The calculation is based on the fact that vitamin B_{12} contains one atom of cobalt in the molecule (about 4% of the mass). For calculating the mean proportion of the total cobalt that was tied up in vitamin B_{12} in the lake, the volumes of water in the various layers should be taken into account. In Table 7 the water volumes calculated from the topographic map of Riley (1939) are given. If the calculated

TABLE 6. *The proportion of the total cobalt at various depths in Linsley Pond tied up in vitamin B_{12}*

Depth m	Cobalt ppb	Vitamin B_{12} ppb	B_{12} as Co ppb	B_{12}Co: total Co per cent
0	0.042	0.075	0.0028	6.7
2	0.021	0.075	0.0028	13.3
3.5	0.025	0.060	0.0022	8.8
5	0.067	0.060	0.0022	3.3
8	0.105	0.065	0.0024	2.3
12.5	0.053*	0.150	0.0056	10.5

* Samples from 11 and 14 meters on 14 August 1951 contained 0.160 and 0.130 ppb cobalt. This suggests that the percentage given for the deepest water in the last column may be too high by a factor of about 3.

TABLE 7. *Volumes of water in the various layers of Linsley Pond*

Characteristic depth m	Interval or layer limits m	Volume m^3	
0	0–1	89,100	
2	1–2.75	134,400	
3.5	2.75–4.25	103,550	
		Epilimnion	327,050
5	4.25–6.50	132,400	
8	6.50–10.25	123,975	
12.5	10.25–14	45,275	
		Hypolimnion	301,650
		Total	628,700

volumes are used as weighting factors in averaging the characteristic percentages (last column of Table 6), the means that result are 10.1 % for the epilimnion and 4.0 % for the hypolimnion. This latter mean may be even lower because of the possibility that deep water is ordinarily richer in cobalt than it was in August 1952. The more efficient conversion of cobalt to vitamin B_{12} in the epilimnion can be ascribed to the activities of blue-green algae in the photic zone. Synthesis by the bacteria in the deepest waters of the lake can account for the high vitamin B_{12} content of the water just over the mud. The plankton on 14 October 1952 was very rich in zooplankton, poor in phytoplankton. Diatoms were absent. The dominant phytoplankters were *Anabaena planktonica*, *A. circinalis*, and a very fine species of *Oscillatoria* or *Phormidium*. The only other common phytoplankter was *Eudorina* sp. Of the blue-green algae known to be capable of vitamin B_{12} synthesis, only a *Diplocystis*, which the writer believes is *D. incerta*, was observed.

The chrysomonad, *Ochromonas malhamensis*, grows only poorly unless the culture medium contains at least 4.0 ppb of vitamin B_{12} (Marston 1956). Less than a tenth of this concentration was found at all depths in Linsley Pond. In fact less than 0.1 ppm was found at all depths except just over the mud. This phytoflagellate would make almost no growth in Linsley Pond water in the summer at least. The extent to which the algal flora is restricted by the very limited supply of vitamin B_{12} can only be guessed at, but the most recent literature shows more and more algal species which, when grown in pure culture, exhibit a need for vitamin B_{12}. The most obvious basis for the low vitamin B_{12} content of Linsley Pond water is the limited cobalt supply. Holm-Hansen *et al.* (1954) gave the optimum cobalt content of mineral culture media for blue-green algae including the bloom form, *Diplocystis aeruginosa*. The cobalt requirement of 0.40 ppb was spared by as little as 0.075 ppb vitamin B_{12}. This suggests an efficiency of conversion of only 0.75 %, which is considerably lower than the efficiency estimated for the photic zone of Linsley Pond. The prolific growths of *Diplocystis* observed in lakes contaminated with sewage may be due to the high vitamin B_{12} content of sewage (Hoover *et al.* 1951) rather than its mineral or trace metal content. The simple field experiment of adding a few grams of cobalt salts (vitamin B_{12} is far too expensive) to a small unproductive pond is suggested. This is precisely what is done in cobalt-poor grazing land to prevent the ruminant deficiency disease.

SUMMARY

The cobalt content of some surface waters in Connecticut was determined colorimetrically. Cobalt, copper, and iron data are given for a vertical series of water samples from Linsley Pond during the summer stratification. The mean contents of cobalt, copper, and iron for Linsley Pond were 9.1×10^{-5} mg/L, 3.4×10^{-3} mg/L, and 1.2 mg/L, respectively. The cobalt value is in the general range of the lowest concentrations reported in the literature. Cobalt and iron, especially the soluble fractions, were greater in the hypolimnion than in the epilimnion. The significance of the distinction between seston and soluble fractions is subject to a strong reservation based on technique in the present work. Theory indicates that there should be no detectable ionic copper in sulfide water. The easily detected quantities of "soluble" copper in the deep water of Linsley Pond were probably in the form of soluble complexes or fine particulate matter.

Linsley Pond seston is estimated to con-

tain about 20 ppm Co, 500–5000 ppm Cu, and 3.5% Fe on a dry basis. The iron content is about the same as the content in most soils, the cobalt is somewhat higher, but the copper content is vastly higher. The high copper content of seston suggests that filter-feeders have a special mechanism to cope with copper in the diet, either selective filtration or selective absorption, or some other mechanism.

Vitamin B_{12} was determined in a vertical series of samples from Linsley Pond. The average content of the water was 0.08 ppb. This is less than one-tenth of the optimal concentration for the chrysomonad, *Ochromonas malhamensis*. The proportion of the total cobalt in the water which was tied up in vitamin B_{12} was between 2% and 13%. The proportion was higher in the epilimnion suggesting that synthesis of the vitamin by blue-green algae in the photic zone is more efficient than by bacteria in the hypolimnion.

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Primary Organic Productivity of a Hawaiian Coral Reef[1]

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ABSTRACT

Primary organic productivity was determined by measuring changes in oxygen concentration of sea water flowing over a fringing coral reef at Kapaa, Kauai, Hawaii. Prevalence of a rather strong, unidirectional current facilitated use of this method.

Determinations were made both in summer (July) and winter (November). The reef is autotrophic, as were two previously studied atoll reefs in the Marshall Islands. Gross primary productivity is about 2,900 g C/m²/yr. This is in impressive agreement with results obtained on the atoll reefs, which are the only previous measurements of coral reef productivity.

All three results are considerably higher than determinations of productivity of open ocean waters. The authors attribute this difference to photosynthesis by benthic algae on the coral reef platform.

INTRODUCTION

The attention of many oceanographers has in recent years been directed toward measurement of rates of biological productivity of the oceans. Riley (1941) summarized data available at that time, most of which had been obtained from the North Atlantic Ocean. More recently, studies of primary organic productivity have been extended to other parts of the open ocean (Steemann Nielsen 1952, 1954, and others; see also Fleming and Laevastu 1956) and to coral reefs.

Prior to the investigation reported in this paper, the only definitive data on coral reef productivity were those provided by Sargent and Austin (1949, 1954) and Odum and Odum (1955) for atoll reefs in the Marshall Islands.

The present paper provides comparable data for a fringing reef in the Hawaiian Islands. It will be shown that (1) the rate of organic production on the reef is sufficient to meet the needs of the consumer organisms present, and (2) the rate of gross primary productivity is of the same order of magnitude as those reported in the two Marshall Islands studies. These rates are considerably higher than those reported for open oceans and, with one exception, somewhat higher than those reported for even the most productive coastal waters. An explanation is advanced to account for this discrepancy.

Location and description of the reef

The investigation was carried out on North Kapaa Reef, at Kapaa, on the windward (east) coast of the island of Kauai (Fig. 1). The reef was visited in October and November of 1955, and in July, 1956.

North Kapaa Reef extends for about 4,000 feet fringing an approximately north-south shoreline (Fig. 2). Its southern boundary is a small stream and stone jetty. A small embayment of deep water which approaches a steeply sloping shore marks its northern limit. The reef reaches a maximum width of 1,400 feet near its southern end and gradually narrows to 500 feet at the northern end.

[1] Contribution No. 92, Hawaii Marine Laboratory. This research was supported by grants from the Society of the Sigma Xi, the Hawaiian Academy of Science, and the Lihue Plantation Co., Ltd. Appreciation is expressed to W. J. Newhouse for surveying the algal flora; to T. S. Austin, G. E. Hutchinson, and G. A. Riley for criticism of the manuscript; to D. C. Cox and F. E. J. Fry for helpful discussion; to M. S. Adachi for preparation of the figures; and to W. J. Atkinson for technical assistance.

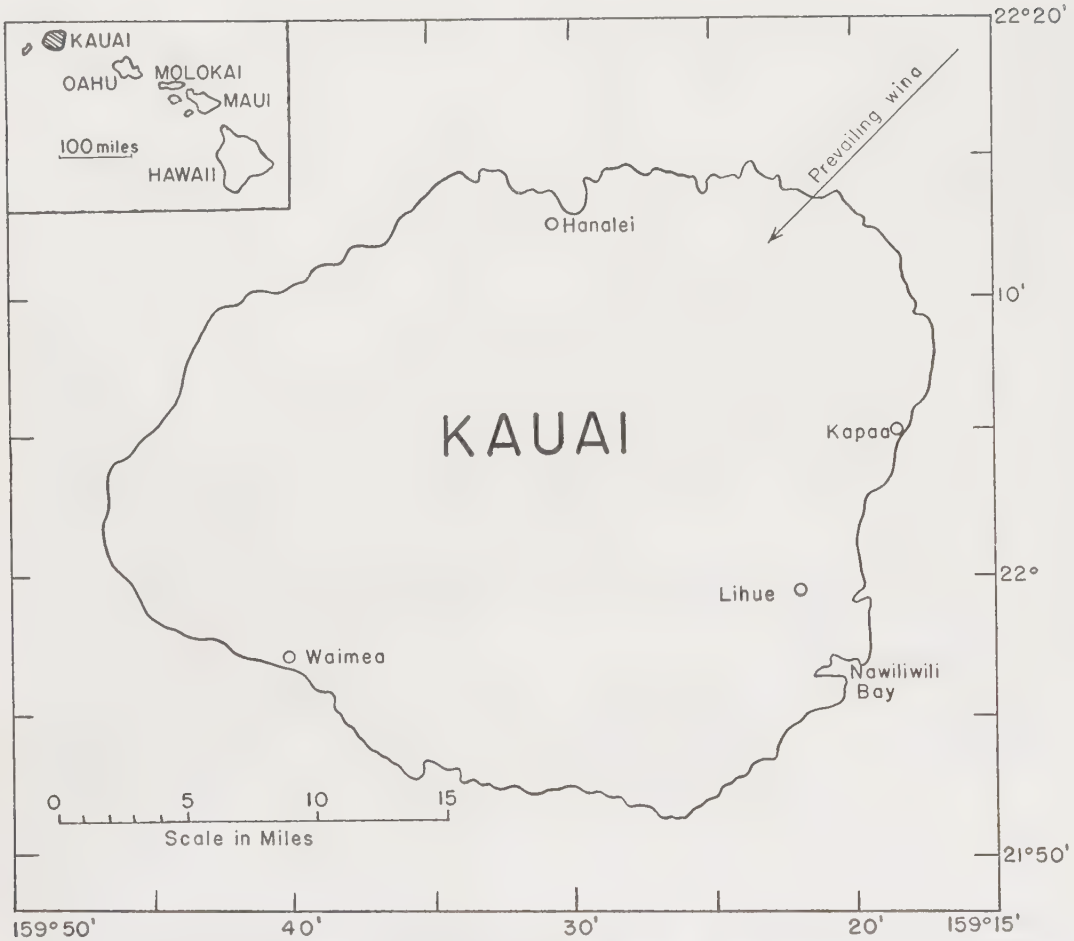

FIG. 1. Outline map of Kauai. The reef studied is at Kapaa. Inset map shows position of Kauai (hatched) in the Hawaiian Islands.

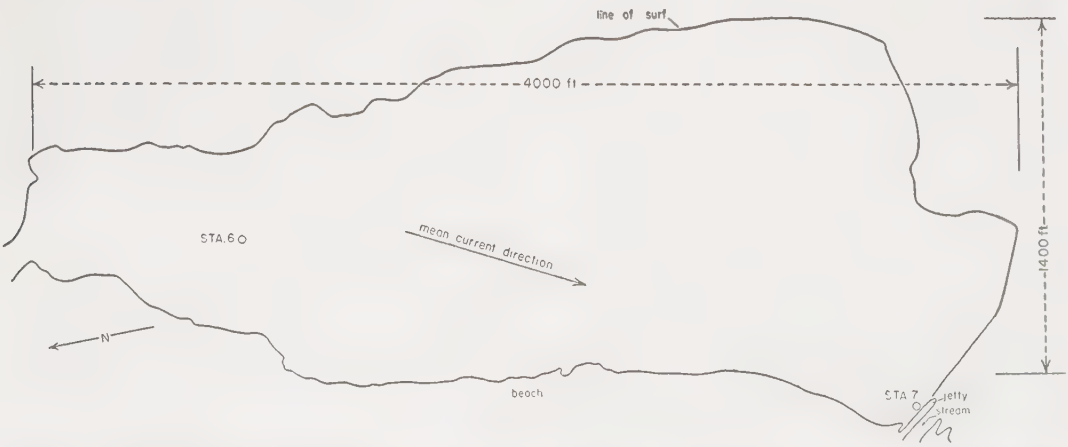

FIG. 2. Outline map of North Kapaa Reef, showing stations, mean current direction, and important topographic features.

Depth of water over the reef varies from 6 to 173 cm above mean sea level. Mean depth was calculated to be 79 cm. This datum represents the average of 107 measurements of depth with respect to mean sea level made by the Lihue Plantation Co., Ltd.

The substratum is characterized by irregular areas of living and dead coral, coral rubble, and sand. The distribution and abundance of benthic organisms vary with the nature of the substratum. A detailed survey of the flora and fauna was not made; however, the general distribution and abundance of prominent organisms were noted (Helfrich and Kohn 1955). Benthic algae, which are of special interest, are represented chiefly by the genera *Acanthophora*, *Lyngbya*, and *Padina*. Other genera of large plants present are *Ulva*, *Halimeda*, *Sargassum*, and *Amansia*. The encrusting *Porolithon*, *Goniolithon*, and *Lithothamnion* occur especially toward the seaward edge of the reef.

Investigations of currents over the reef revealed a definite pattern. A rather strong, unidirectional current prevails. Fifty-two determinations in October and November of 1955 gave a mean current velocity of 24 cm/sec and a mean heading of 220°T. Twenty determinations in July, 1956, gave a mean velocity of 22 cm/sec and a mean heading of 218°T. The current is thus approximately parallel to the coastline and to the prevailing northeast trade winds. At the southern end of the reef the current is deflected out to sea by the jetty (Fig. 2) and reached a maximum velocity of 44 cm/sec. (Measurements of currents at this station [Sta. 7] were not included in average velocities and headings.)

Vertical diurnal tide range is only about 1.8 feet in the area under consideration. Calculations made for the authors by D. C. Cox, Senior Geologist, Hawaiian Sugar Planters Experiment Station, showed that the volume of water moved on and off the reef with change in tidal phase does not cause a significant tidal current. This was verified by plotting observed current velocities against time and tidal phase (Fig. 3). These graphs show significant increases in mean

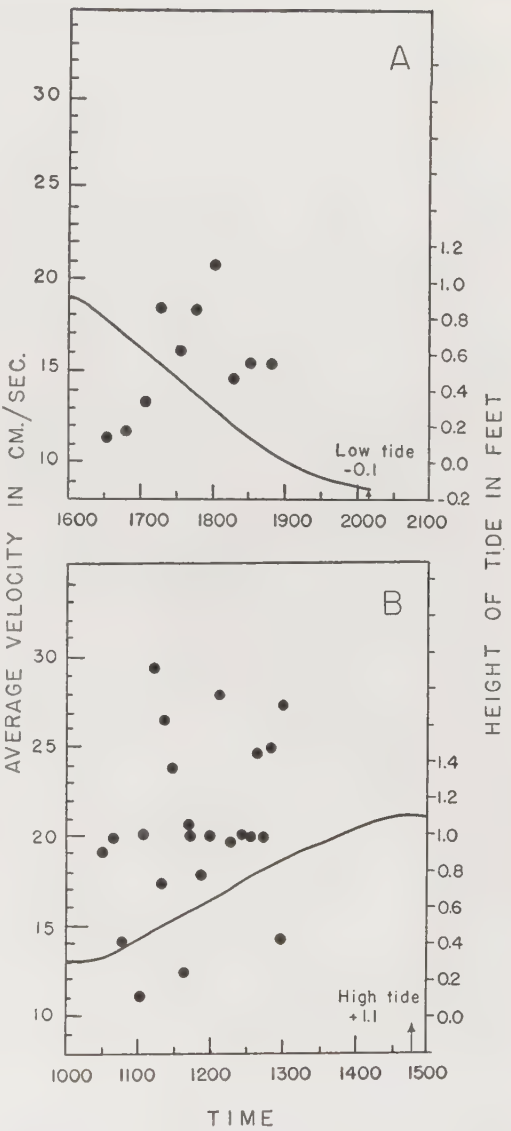

Fig. 3. Individual determinations of current velocity (●), and height of tide (——), plotted against time of day, showing absence of relationship between current velocity and phase of tide. *A*, 29 October 1955. *B*, 30 October 1955.

current velocity with time; however, it should be noted that the determinations on 29 October were made while the tide was falling, while those on 30 October were made during rising tide.

METHODS

Two stations (Sta. 6 and 7) were established on the reef such that a line drawn

between them was approximately parallel to the mean current direction (Fig. 2). Distance between the stations was 861 meters.

Each of the determinations of current velocity cited above consisted of recording the time required for a fluorescein dye spot to travel a distance of 5 m. Current direction was determined by sighting with a compass from the point of release of the dye spot to its position after traveling 5 m.

Water samples were collected in 300-ml glass-stoppered bottles at intervals over a 24-hour period. Samples were taken at a depth of approximately six inches below the surface. They were fixed immediately for determination of dissolved oxygen by the Winkler method. The samples were kept in darkness and titrated upon return to the laboratory 2–3 days later. Replicate samples of 50 ml were titrated with 0.01 N sodium thiosulfate standardized with potassium biniodate (Harleco).

In the November series, records for a single cycle were obtained. In July, data were obtained for two successive 24-hour cycles.

Water temperature at each station (Fig. 4A) and incident light intensity (Fig. 4B) were measured simultaneously with collection of each water sample in the July series. A Norwood Director exposure meter placed on a level surface on the shore near Sta. 7 was used to measure incident light. At times of intermittent cloudiness series of readings were taken and averaged. Meter readings were converted to foot-candles by multiplication by the appropriate factor. Data on cloud cover (Fig. 4B, 5A) as well as other weather data were obtained from the Weather Bureau Pacific Supervisory Office in Honolulu, through the courtesy of D. I. Blumenstock. All Weather Bureau observations were made at Lihue, Kauai, approximately ten miles south of Kapaa.

RESULTS

Observations of 27–29 July 1956

In the July series of observations an effort was made to test the methods used by comparison of productivity during two successive 24-hour cycles. The results are summarized in Figure 4. Water temperature fluctuated diurnally at both the upstream station (Sta. 6) and the downstream station (Sta. 7), as shown in Figure 4A. Maximum and minimum temperatures and diurnal ranges at both stations are given in Table 1. The greater amplitude of fluctuation at Sta. 7 is attributed to the increased surface/volume ratio of the water as it flows from the open ocean across the shallow reef platform.

Measurements of incident light and the extent of cloud cover are shown in Figure 4B.

Results of oxygen determinations at each station are shown graphically in Figure 4C. At both stations a diurnal cycle of oxygen concentration is apparent. The greater amplitude at Sta. 7 is attributed to biological activity on the reef platform.

The mean observed current velocity in July was 22 cm/sec. The time required for water to pass from Sta. 6 to Sta. 7 was close to one hour (3910 sec.). Therefore, the curve for Sta. 6 in Figure 4C has been shifted to the left by one hour, according to the convention suggested by Odum (1956). This enabled analysis of the data in such a way as to make possible the determination of rates of primary organic production (photosynthesis) and consumption (respiration) on the reef. This method was first used by Sargent and Austin (1949, 1954).

Since the mean compass bearing of Sta. 7 from Sta. 6 was 218°T. and the mean current direction was 220°T., the two stations utilized were virtually on a line coinciding with the mean current direction, and no correction for angle of current was necessary.

The mean rate of change of oxygen concentration in milliliters per centimeter of reef normal to the current (*i.e.* in a strip 1 cm wide between the two stations) is given by the formula

$$\text{mean depth (cm)} \times \text{velocity (cm/sec)} \times \text{mean change in oxygen between stations (ml/cm}^3\text{)} = \text{mean change in oxygen over reef (ml/cm/sec)} \quad (1)$$

Substitution in Equation (1) of observed values for mean depth and mean current velocity gives

$$79 \times 22 \times (\text{ml/L}_{\text{Sta. 7}} - \text{ml/L}_{\text{Sta. 6}}) \times 10^{-3} = \text{ml/cm/sec}. \quad (2)$$

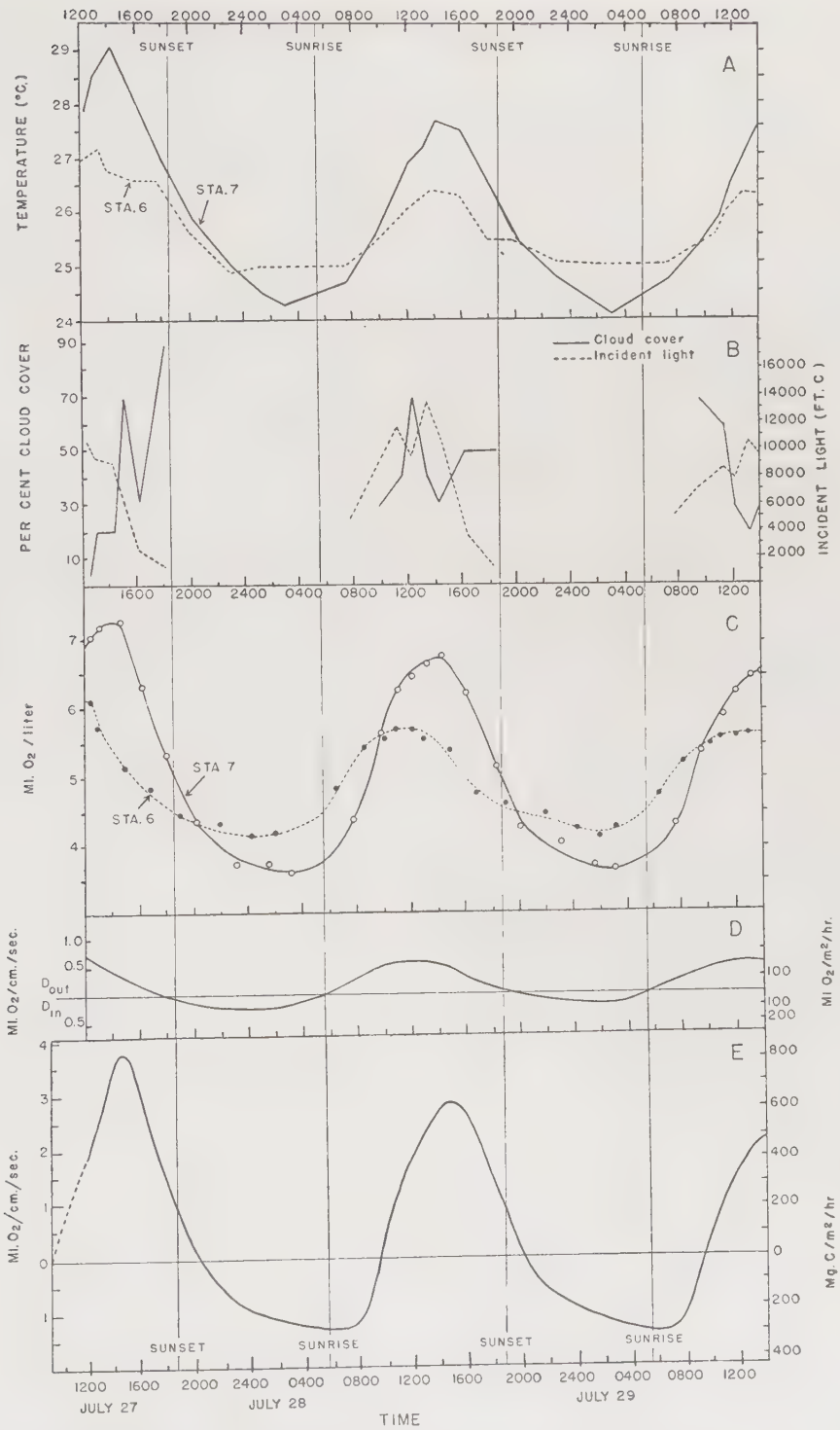

FIG. 4. *A*, Sea surface temperatures at Sta. 6 and 7. *B*, Incident light and cloud cover. *C*, Oxygen concentration at Sta. 6 and 7. *D*, Diffusion of oxygen across the air-sea interface. *E*, Production and consumption of organic matter. Data from North Kapaa Reef, 27–29 July 1956.

TABLE 1. *Temperature (°C) maxima, minima, and ranges at stations 6 (upstream) and 7 (downstream)*

	Maximum		Minimum		Range	
	Sta. 6	Sta. 7	Sta. 6	Sta. 7	Sta. 6	Sta. 7
27–28 July	27.2	29.1	24.9	24.3	2.3	4.8
28–29 July	26.4	27.1	25.0	24.1	1.4	3.0

As emphasized by Odum (1956) the curve of this equation is not equivalent to the difference between rates of gross primary productivity and respiration but includes also the result of exchange of oxygen with air.

A convenient method of correcting for diffusion of oxygen across the air-sea interface is presented by Odum (1956) in the following equations:

$$D = KS \tag{3}$$

where D is the diffusion rate per area, K is the gas transfer coefficient defined on an area basis, and S is the saturation deficit between water and air.

$$K = \frac{z(q_m - q_e)}{S_m - S_e} \tag{4}$$

where z is the mean depth, q_m is the rate of change of oxygen concentration before sunrise, q_e is the rate of change of oxygen concentration after sunset, S_m is the saturation deficit before sunrise, and S_e is the saturation deficit after sunset. Since respiration is assumed to be constant it does not affect q.

The gas transfer coefficient K is also known as the invasion coefficient (Dorsey 1940), and the exchange coefficient, E (Redfield 1948). The only values which have been calculated for the oceans are those given by Redfield (1948) for the Gulf of Maine. The coefficient in winter was 13×10^6, and in summer, 2.8×10^6 ml $O_2/m^2/month/atmosphere$. The value calculated for North Kapaa Reef is 3.3×10^6 ml $O_2/m^2/month/atmosphere$. Redfield's calculations were based on saturation values given by Fox (1909), which have since been superseded by those of Truesdale, Downing, and Lowden (1955). Use of the data of Fox's tables in the calculation for North Kapaa Reef would increase K by about 15%.

In order to plot the diffusion curve (Fig. 4D) in the same units as Equation (2), z is in meters, q_m and q_e in $ml/m^3/hr$, and S_m and S_e are expressed as the head of oxygen pressure across the sea surface, in atmospheres, calculated from the formula given by Redfield (1948: p. 355) using the saturation data of Truesdale, Downing, and Lowden (1955). Thus K may be expressed in ml/cm/sec/atm. Since S, like S_m and S_e, is in atm, D is in ml/cm/sec. The cm in this expression, as in Equations (1) and (2), refers to a strip of reef 1 cm wide between the two stations, which is equivalent to an area of 8.6 m^2. Diffusion may thus be converted to an areal basis, as has been done on the right ordinate of Figure 4D.

The curve of Equation (1) corrected for diffusion by algebraic addition of the diffusion curve (Fig. 4D) is plotted as Figure 4E. The portion of the curve above the zero or compensation line represents an excess of primary organic production over consumption. The portion below the line represents net consumption.

The nocturnal level of respiration is of interest. The difference between stations during the night decreases to a minimum of about -1.4 ml O_2/cm/sec near sunrise (or shortly after, suggesting a lag). This represents nocturnal respiration by all of the organisms on the reef, and it may be used as an indication of respiration of the entire biological community on the reef. The area of a rectangle bounded by 0 and -1.4 ml O_2/cm/sec on the ordinate of Figure 4E and extending for 24 hours on the abscissa is equivalent to 121,000 ml of oxygen consumed per cm (= 8.6 m^2) per 24-hour day, or 14,100 $ml/m^2/day$.

Since clear evidence to the contrary is lacking, it is simplest to assume that the rate of plant respiration is independent of the rate of photosynthesis (Odum 1956, Ryther 1954). Therefore, the consumption of 14,100 ml of oxygen per square meter per day may be taken as the respiration rate of the entire community, and it reflects quantitatively the biomass present. This datum is introduced here with the suggestion of possible future correlations between measurements of biomass and rates of metabolism of biological communities.

TABLE 2. *Net production and consumption in ml O_2/cm on North Kapaa Reef, 27–29 July 1956*

Based on graphical integration of areas above (production) and below (consumption) 0-line in Figure 4E.

	Net production	Net consumption
27–28 July	73,700	45,600
28–29 July	64,600	46,700

In order to compare net production and consumption on the reef, the curve of Figure 4E was graphically integrated. Values for net production (area above 0 line) and net consumption (area below 0 line) are given for the two days studied in Table 2. Inspection of Table 2 and Figure 4E shows reasonable similarity of the data for successive days, thus affirming the validity of the method. The first sample collected was at 1200 on 27 July. In order to obtain a complete cycle for this date, the time of the 0 intercept was taken as the average (0916) of the observed times on 28 July (0920) and 29 July (0912). For this reason, the portion of the curve for 27 July between 0916 and 1200 is shown as a dotted line in Figure 4E. Mean net production was about 69,200 ml and mean net consumption about 46,600 ml of oxygen for a 24-hour day. There is thus a mean excess of 22,600 ml of oxygen per day.

The rate of primary production of living substance is of greater interest than the rate of exchange of oxygen *per se*. Therefore, in the conversion to areal units on the right ordinate of Figure 4E, milliliters of oxygen have been converted to milligrams of carbon by the simplified photosynthesis equation,

$$CO_2 + H_2O = (CH_2O) + O_2 \qquad (5)$$

The daily excess of 22,600 ml of oxygen is equivalent to 12,100 mg of carbon, by which production exceeds consumption per centimeter of reef normal to the current per day. In areal units, the excess is about 1,400 mg C/m²/day.

Production averaged 4,300, and consumption 2,900 mg C/m²/day, giving the production excess of 1,400 mg C/m²/day. In summer, a significantly greater amount of organic matter is thus produced on the reef over that which is consumed. Thus, the reef is self-sufficient, or autotrophic, at least during this part of the year. The excess is presumably stored on the reef as organic biomass.

In order to determine gross primary productivity, the entire curve (Fig. 4E) above the baseline (−1.4 ml O_2/cm/sec) was graphically integrated. This represents 133,000 ml O_2/cm/day for the only complete cycle covered by the observations (28 July). This is equivalent to about 71.3 g C/cm/day, or 8.3 g C/m²/day. The ratio of gross primary productivity to community respiration (P/R) is 1.1.

If this rate of production were maintained throughout the year, annual productivity would be 3,000 g C/m².

Observations of 25 November 1955

The results of the initial study, made 25 November 1955, are summarized in Figure 5. Temperature measurements taken at this time were too few to permit construction of curves of diurnal fluctuation and diffusion. Those obtained were about 1.5°C lower than corresponding values in July.

Incident light measurements were not made in the November series, but the extent of cloud cover for the period under consideration has been plotted in Figure 5A. In the field notes it was stated that the "data were collected on a day throughout which the sun shone brightly."

Results of determinations of oxygen concentrations at each station are shown graphically in Figure 5B. The diurnal cycle at both stations is apparent, as is the greater amplitude of the curve for Sta. 7, which is expected from increased biological activity on the reef platform.

The mean observed current velocity in November was 24 cm/sec. Time required for water to pass from Sta. 6 to Sta. 7 was one hour (3590 sec). Therefore, the curve for Sta. 6 in Figure 5B has been shifted to the left one hour. Since the mean current direction was close to the compass bearing of Sta. 7 from Sta. 6, correction for angle of current was not necessary.

The rate of change in oxygen concentra-

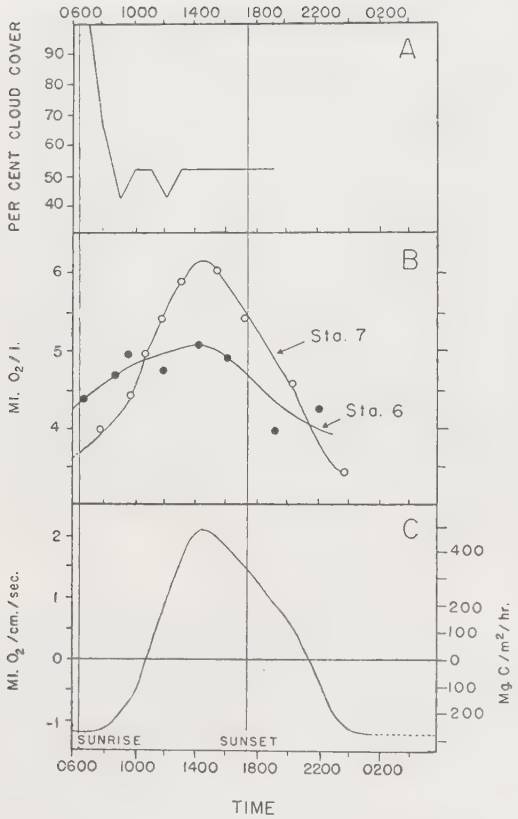

FIG. *A*, Cloud cover. *B*, Oxygen concentration at Sta. 6 and 7. *C*, Production and consumption of organic matter (not corrected for diffusion). Data for North Kapaa Reef, 25 November 1955.

tion between stations throughout the 24-hour cycle is plotted in Figure 5C. This graph is not directly comparable with Figure 4E, since the latter has been corrected for diurnal fluctuation of diffusion, while the former has not.

The nocturnal level of respiration in Figure 5C is about −1.2 ml/cm/sec. Since diffusion is in the direction of air to water at night, its effect is to reduce the apparent difference in concentration between Sta. 6 and Sta. 7. Thus the observed rate of respiration is probably too small. The actual rate probably approaches closely the July rate of −1.4 ml/cm/sec, or consumption of 14,100 ml/m²/day.

Graphic integration of the curve (Fig. 5C) gives uncorrected values of 47,100 ml O_2/cm/sec net production and 46,900 ml O_2/cm/sec net consumption. In the July series of observations, correction for diffusion resulted in increasing net productivity by 20.7% and increasing net consumption by 11.5%. If this discrepancy applies also in winter, daily production is probably somewhat greater than daily consumption. Thus in winter as well as in summer, sufficient organic matter is produced on the reef to meet the demands of the consumer organisms present.

Gross productivity, as calculated from the uncorrected curve (Fig. 5C) is 103,100 ml O_2/cm/day. In the July series, correction for diffusion raised the calculated gross productivity by 19.5%. Application of this factor to the November result gives a corrected gross productivity of 123,200 ml O_2/cm/day. This is equivalent to 7.7 g C/m²/day, somewhat lower than the 8.3 g/m²/day for July.

If the calculated summer and winter values are each assigned one-half year, annual gross primary productivity is calculated to be 2,900 g C/m².

Efficiency of gross production

Determinations of solar radiation made by the writers were not sufficiently numerous to support an estimate of photosynthetic efficiency. Records of insolation obtained at the Hawaiian Sugar Planters Association Experiment Station in Honolulu indicated 7.15×10^6 cal/m²/day for 30 July 1956. Using the energy equivalent of the July production datum of 8.3 g C/m²/day, efficiency of gross productivity was calculated to be 1.1%. This is lower than the value of 5.8% measured on an atoll reef (Odum and Odum 1955) but somewhat higher than other marine communities. Riley (1941) calculated values of 0.55–0.82% efficiency for phytoplankton in Long Island Sound.

DISCUSSION

In previous quantitative determinations of primary productivity of coral reefs, no corrections for diffusion of oxygen have been made. For this reason, the directly comparable uncorrected curve for North Kapaa Reef are shown in Figure 6. In Figure 6A,

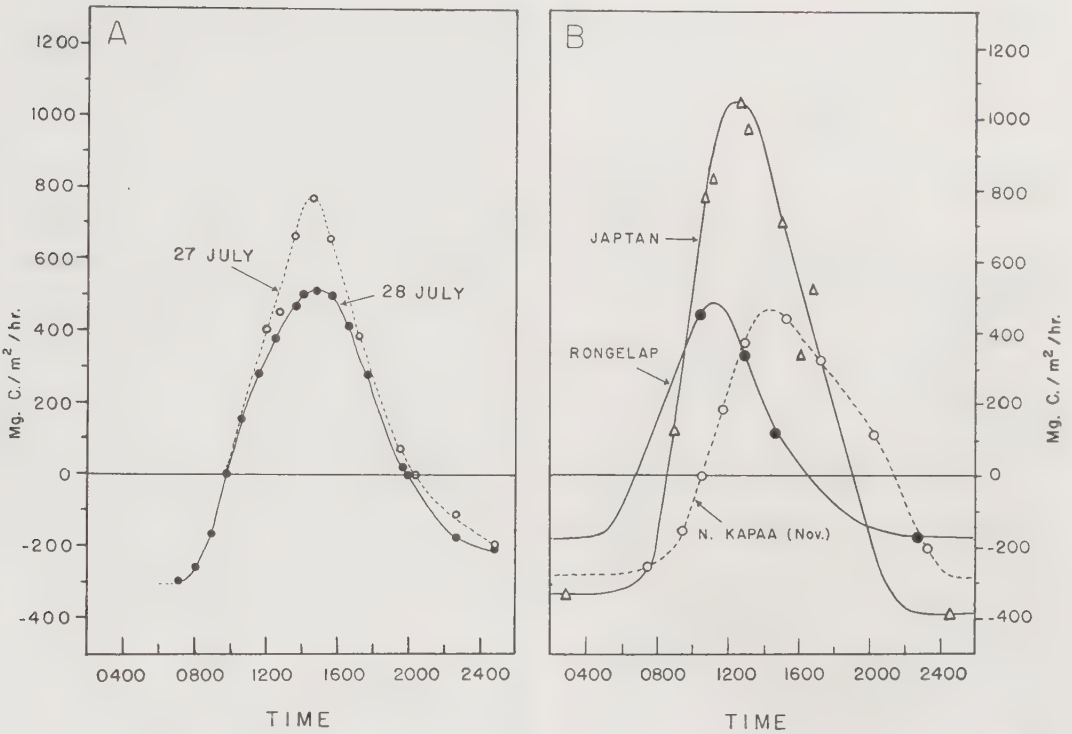

FIG. 6. Production and consumption of organic matter. *A*, North Kapaa Reef, 27 July 1956 and 28 July 1956. *B*, North Kapaa Reef, 25 November 1955; reef at Japtan Island, Eniwetok Atoll; and reef at Bokujarito Island, Rongelap Atoll. Data not corrected for diffusion. Data from Japtan and Bokujarito are from Odum and Odum (1955) and Sargent and Austin (1954), respectively.

the two cycles observed on North Kapaa Reef in July have been superimposed to facilitate comparison. The curves obtained by Sargent and Austin (1949, 1954), Odum and Odum (1955), and for North Kapaa Reef in November have been recalculated in terms of mg C/m²/hr and included in Figure 6B.

It is noteworthy that the peaks of production for the three determinations made on North Kapaa Reef (27 and 28 July and 25 November) are in very close agreement. All three fall between 1400 and 1430. They are later, however, than those obtained by Sargent and Austin (1949, 1954) at Rongelap (before noon) and by Odum and Odum (1955) at Japtan (about 1230). Sargent and Austin (1954) point out that a morning peak is reasonable in the light of results obtained by Kostytschew *et al.* (1926, 1930), who observed that the rate of photosynthesis both in land and water plants reached a maximum before noon and then declined. Yonge, Yonge, and Nichols (1932) also observed this phenomenon in reef corals.

Odum and Odum (1955) explain that the lag between sunlight intensity and oxygen production may be partially due to location of photosynthesizing organisms beneath the surface of the calcareous reef surface, sand, and coral polyps. This also seems the best explanation for the occurrence of a lag in peak production at North Kapaa Reef. However, it does not explain why the peak at Kapaa is later than at Japtan. The discrepancies in time of peak production in these investigations seems sufficient to suggest that this aspect requires further investigation, especially with regard to the extend of oxygen production and its rate of diffusion from primary producers within coral and other types of substratum.

The three coral reefs that have been studied all show a daily excess of production of organic matter over consumption.

TABLE 3. *Gross primary organic productivity of marine environments*

Location	Gross productivity g C/m²/yr	Reference
Coral Reefs		
Rongelap Atoll, Marshall Is.	1500 (1800)[1]	Sargent and Austin (1954)
Eniwetok Atoll, Marshall Is.	3500 (4200)[1]	Odum and Odum (1955)
North Kapaa Reef, Kauai, Hawaii	2900	Present paper
Turtle Grass Bed		
Long Key, Florida	4650	Odum (1956)
Open Ocean		
Off Rongelap Atoll, Marshall Is.	28	Sargent and Austin (1954)
Off Hawaii	37	Steemann Nielsen (1954)
Off Hawaii[2]	21	Doty and Oguri (1956)
Off Hawaii (Inshore waters)[3]	123	Doty and Oguri (1956)
Sargasso Sea	167	Riley, Stommel, & Bumpus (1949)
Georges Bank	309	Riley, Stommel, & Bumpus (1949)
Long Island Sound	470	Riley (1956)
Benguela Current	167–912	Steeman Nielsen (1954)

[1] With estimated correction for diffusion.
[2] Average of Stations 1 and 2.
[3] Station 3.

Sargent and Austin (1954) reported net production of 0.5 g C/m²/day, and Odum and Odum (1955) 9 g C/m²/day. The mean net production in the present study, corrected for diffusion, is 7.9 g C/m²/day.

In the second column of Table 3, the annual values of gross productivity reported by Sargent and Austin (1954) and Odum and Odum (1955) are listed. These values were not corrected for diffusion. For North Kapaa Reef, the diffusion correction represents an increase of 19.5%. This factor has been applied to the Marshall Islands data to give an estimate of corrected gross productivity. These values are placed in parentheses in Table 3.

Two important aspects of these data merit further discussion. First, the present authors are impressed by the fact that all three of the coral reefs thus far studied show gross productivity values of the same order of magnitude, when determined by the flow method. The highest value differs from the lowest by a factor of about two. Sargent and Austin (1954) described the reef at Rongelap as appearing "rather barren of plants." The rich biota of Japtan Reef, Eniwetok Atoll, is described in detail by Odum and Odum (1955). Standing crop on North Kapaa Reef, although not quantitatively determined, may well lie in between the other two reefs.

Second, these values are one to two orders of magnitude greater than those reported for almost all other marine environments. Data from other areas are included for comparison in Table 3. Excluding the coral reef and turtle grass communities, the highest values are for Long Island Sound, a shallow, protected body of water characterized by a nutrient-rich, freshened surface layer, and for the southern part of the Benguela current. The latter is a locality of marked upwelling. A single experiment gave a rate equivalent to 1387 g C/m²/yr, but considerable variation was expected (Steemann Nielsen 1954). In all of the other regions cited, with the probable exception of Doty and Oguri's (1956) Sta. 3, the base of the euphotic zone is above the ocean floor. In contrast, the shallow coral reef platforms support more or less dense populations of benthic algae. Productivity due to phytoplankton of neighboring oceanic water is extremely low. It is apparent that the extremely high gross primary productivity of coral reefs is attributable to photosynthesis by benthic algae on the reef platforms.

The extensive substrate area covered by a shallow layer of constantly flowing sea water is concluded to be the major factor responsible for the productivity of coral reefs being two orders of magnitude higher than adjacent oceanic waters. The substrate enables the growth of large attached algae, as well as algae which live within the substratum in a strongly illuminated region of the sea. In some regions, attached phanerogams play an important role. The strong constant flow of water provides these plants with a continual source of nutrients. The plants in turn promote a rich community of large and small animals confined to the same surface. Communities of adjacent areas may also benefit, as indicated by higher phytoplankton productivity of Hawaiian inshore waters (Doty and Oguri 1956).

The highest gross primary productivity

value reported for a marine community is 4650 g C/m²/yr (Odum 1956). It was obtained at a depth of three feet in a bed of turtle grass (*Thalassia*), which is undoubtedly the primary source of this productivity.

SUMMARY

Primary organic productivity was determined by measuring changes in oxygen concentration of sea water flowing over a coral reef at Kapaa, Kauai, Hawaii. Prevalence of a rather strong, unidirectional current facilitated use of this method. Determinations were made both in summer (July) and in winter (November).

The reef is autotrophic, that is the rate of organic production is sufficient to meet the requirements of the consumer organisms present. This was also found to be the case on two atoll reefs in the Marshall Islands, on which studies of this type had previously been made.

Gross primary productivity was calculated to be 2,900 g C/m²/yr. This is in impressive agreement with data obtained by Sargent and Austin (1954) and Odum and Odum (1955) on the atoll reefs.

The ratio of gross productivity to community respiration was calculated to be 1.1. Efficiency of gross productivity was calculated to be 1.1%.

Except for a rather specialized turtle grass community, the primary productivity of coral reefs is far greater, on an areal basis, than all other marine environments that have been studied. The authors attribute this difference to photosynthesis by benthic algae on the reef platforms.

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Phytoplankton of the North Central Sargasso Sea, 1950–52[1]

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ABSTRACT

U. S. Weather Ship personnel operating at Lat. 35°N, Long. 48°W obtained approximately weekly collections of water samples for phytoplankton enumeration at five depths between 0 and 150 m for a two-year period beginning in February, 1950. Temperature and Secchi disk measurements and zooplankton collections also were made. The average concentration of phytoplankton was sparse, but it extended through a long vertical column, in agreement with previous observations. There was a diatom increase in April of each year, which appeared to be analogous to the vernal augmentation in temperate waters although of miniature size. The seasonal minimum occurred in autumn, and the population tended to be larger in winter than in summer. Destruction of the seasonal thermocline between December and March presumably increased the rate of nutrient supply at that season, but light and turbulence were critical factors. Hence the development of winter diatom pulses was correlated with the stability of the water column. The species composition was mainly subtropical or ubiquitous, but occasional localized concentrations of temperate-water diatoms suggested eddy transfer from the north. Analysis of previous oceanographic observations in the region by three independent methods indicated an average daily rate of net phytoplankton production of about 0.15 g carbon per m² of sea surface, or a quarter to a third of the observed rates in highly productive temperate waters.

INTRODUCTION

During a two-year period beginning in February, 1950, U. S. Coast Guard personnel obtained plankton collections at approximately weekly intervals at Weather Station B in Davis Strait (56° 30′ N, 51° 00′ W) and at Station E in the Sargasso Sea (35° N, 48° W). The phytoplankton from Station B has been described by Holmes (1956) and the zooplankton by Kielhorn (1952). Fish (1954) has published a preliminary account of the zooplankton at Station E, and the phytoplankton will be discussed herein.

Until a few years ago, our knowledge of plankton cycles in oceanic waters was only what could be pieced together from scattered expedition reports. The American and Norwegian weather ships have performed a unique service to oceanography in obtaining routine collections over considerable periods of time at stations located hundreds of miles from land. The American program was organized and administered by Charles J. Fish and supported in part by the Office of Naval Research. To these the writer is deeply grateful, as well as to the many Coast Guard officers and men who participated in the collections. Thanks are also due Frederick C. Fuglister, Dean F. Bumpus, and other members of the Woods Hole Oceanographic Institution who have been very generous in providing background data, much of it oceanographic information in the Woods Hole files that is as yet unpublished.

METHODS

Water samples of about 400 ml each were obtained with a Nansen bottle at depths of 0, 25, 50, 100, and 150 m, preserved with neutral formalin, and stored in Mason jars. Each station also included zooplankton tows, a Secchi disk reading, and several bathythermograph lowerings. The latter were used to determine the maximum depth of nets and water bottles as well as the temperature distribution.

[1] Contribution No. 17 from the Narragansett Marine Laboratory of the University of Rhode Island. These studies have been aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Rhode Island; reproduction in whole or in part is permitted for any purpose of the United States Government.

Upon arrival in the laboratory, the jars were put on a table with covers loosened and allowed to stand for three days for the phytoplankton to settle. The supernatant water was then siphoned off slowly until 15 ml remained. The plankton concentrate was removed to a vial; the jars were rinsed thoroughly and the washings were added to the vial. The remainder of the siphoned water was measured in a graduated cylinder and discarded. Conover (1956) has examined this method of concentration and has found that the loss of cells is negligible.

The plankton concentrate was later reduced to 2.5 ml. A 1-ml portion was plated out in a Sedgwick-Rafter cell for phytoplankton enumeration. A routine count comprised the following: (a) a cursory examination of the whole chamber for large species that might be important volumetrically although few in number; (b) a count of the identifiable cells in 80 fields at a magnification of 150×, usually equivalent to examining about 10–20 ml of the original water; (c) a count of small nannoplankton of 5–15μ, whether identifiable or not, in three fields. Identifications (all by the present writer) were checked under higher magnification with subsidiary water and Hyrax mounts.

Samples of about 20 cells of each species were measured, leading to rough estimates of volume by application of geometrical formulas for the shapes approximated by the species in question. No attempt was made to achieve a high degree of accuracy. For example a given species was not measured in every sample, but only when it was apparent upon inspection that the size was changing from week to week. Nevertheless, a rough estimate of this sort is quantitatively superior to simple enumeration, since the largest species had ten thousand times the volume of the smallest, and the average volume per cell of the total population varied by more than an order of magnitude during the course of a year.

All of the first year's samples were counted so that a relatively complete picture of vertical distribution was obtained. For purposes of generalization, the mean number $\bar{N}$ of cells in the upper 150 m was computed from the formula

$$\bar{N} = \frac{1}{150}\left[\frac{25(N_0 + N_{25})}{2} + \frac{25(N_{25} + N_{50})}{2} + \frac{50(N_{50} + N_{100})}{2} + \frac{50(N_{100} + N_{150})}{2}\right]$$

where N_0 is the number of cells at 0 m, etc. During the second year of the survey, a single count was made for each collection date. A one-liter composite sample was prepared containing the following quantities of water from each depth; 0 m, 83 ml; 25 m, 167; 50 m, 250; 100 m, 333; 150 m, 167 ml. So prepared, the sample yielded a count equivalent to $\bar{N}$ in the above equation.

THE PHYSICAL AND CHEMICAL ENVIRONMENT

The Sargasso Sea is roughly defined as a subtropical region forming the center of the great North Atlantic eddy. Thus it is bordered on the south by the North Equatorial Current and on the west and north by the Gulf Stream system, while to the east it merges insensibly with the diffuse, southward flowing currents of the eastern North Atlantic. Fuglister and Worthington (1951) on two occasions have located the Gulf Stream at about 39° N at the longitude of Station E, and Iselin (1936) concluded that the southern limit of the Sargasso Sea lies at about 24° N. Thus Station E is in the north central part of the area.

Radiation and transparency

Sverdrup *et al.* (1942) listed average monthly radiation values at a series of latitudes in the western North Atlantic. Interpolation of their data indicates an annual variation in monthly means at Station E from 0.117 g-cal cm^{-2} min^{-1} in December to 0.318 in June. This is about three times the annual variation in the tropics. While the midwinter minimum is by no means as extreme as in higher latitudes, later evidence will indicate that winter phytoplankton growth was sometimes limited by low light intensity.

Figure 1A shows Secchi disk readings routinely obtained at Station E, which are

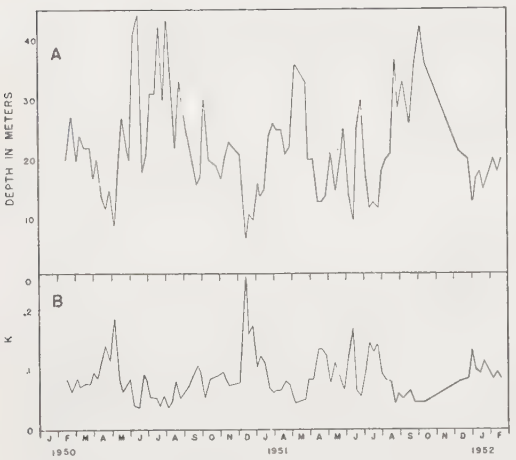

FIG. 1. *A*. Secchi disk readings in meters at Station E. *B*. Extinction coefficient K of visible light, estimated from Secchi disk readings.

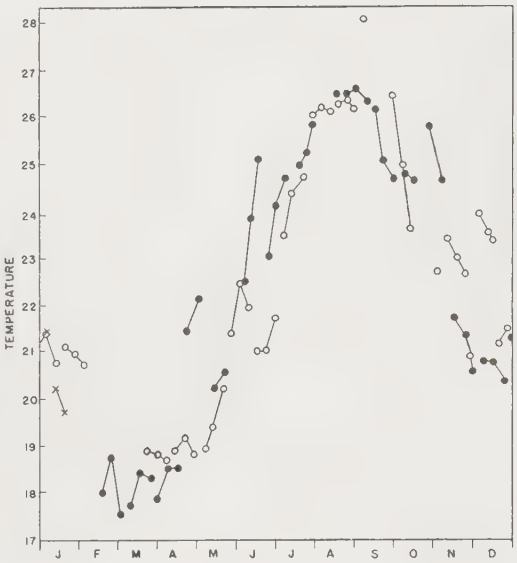

FIG. 2. Surface temperature at Station E plotted as weekly averages of bathythermograph readings. Lines are drawn between observations obtained during a single cruise and with the same instrument. Dots, 1950; circles, 1951; x's, 1952.

transformed in Figure 1B to estimates of the extinction coefficient of visible light according to the Poole and Atkins (1929) formula, $K = 1.7/D$, where K is the extinction coefficient per meter, and D is the Secchi disk reading in meters. Clarke (1939, 1941) has obtained extinction coefficients ranging from 0.042 to 0.072 in various parts of the Sargasso Sea and particularly in the northwestern part. Clarke and others have recorded Secchi disk readings of 18 to 41 m. While a large proportion of the data in Figure 1 fall within these limits, it is apparent that the Sargasso Sea is not always highly transparent.

Temperature

The weather ships routinely collected three bathythermograms per day, which were photographed at the Woods Hole Oceanographic Institution and filed there. Photographs covering the period of plankton collections were read to the nearest 0.1°F at depths of 0, 20, 40, 60, 80, 100, and 140 m, averaged by weeks, and converted to Centigrade scale. In preparing weekly averages, the work of each ship was kept separate. Each cruise ordinarily lasted three weeks, so that three such averages were usually obtained from each vessel.

Figure 2 shows weekly averages for the surface waters. Although a seasonal trend is readily apparent, several groups of data seriously departed from the trend, and the variations between cruises were significantly larger than the variations within a single cruise. The photographic procedure corrected the traces according to the accompanying bucket or injection thermometer readings, so that the latter must have been in serious error.

It was desirable for purposes of later analysis to attempt to smooth the observations into a credible seasonal cycle. At the 140 m level, where seasonal variations were least, it was found that a smooth curve could be drawn through 18 out of a total of 34 cruises. The remainder were arbitrarily fitted into this curve by introducing correction factors into each cruise. The corrections ranged from 1° to −2.4°. The results are shown in Figure 3A, and the data are further condensed in Figure 3B into monthly averages for the two-year period. This is assumed to be adequate for generalizations about the seasonal cycle and for computations of vertical eddy conductivity. However, both the original data and the arbitrarily corrected version are obviously unsuitable for detailed ecological applications. The later discussion will indicate that there is considerable

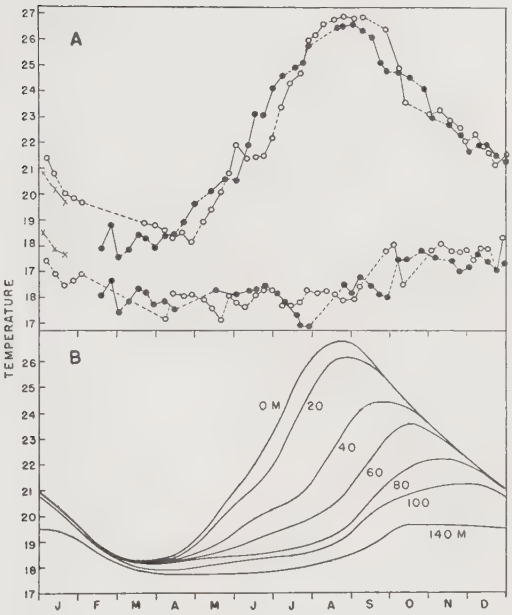

FIG. 3. *A*. Weekly averages of bathythermograph readings at 0 and 140 m, arbitrarily altered to smooth the seasonal cycle. Symbols as in Fig. 2. *B*. Smooth curves drawn through monthly averages for the two-year period of the investigation.

exchange of water masses at Station E, and it is particularly unfortunate that the temperature data are not accurate enough to aid the analysis of these movements.

Examination of hydrographic stations in the central Sargasso Sea indicated that the maximum depth of seasonal variation was 250 to 300 m. The main part of the permanent thermocline lay between 300 and 1000 m. The temperature at the latter depth was 6 to 7°, and there was a further decline to about 3.7° at 2000 m.

Currents

U. S. Hydrographic Office current charts for the North Atlantic Ocean (H. O. Misc., No. 10,688) show surface currents derived from ships' reports and averaged by months for one-degree squares of latitude and longitude. Estimates of mean current velocity in the vicinity of Station E were obtained by taking vectorial averages of the four squares surrounding the station position (34–36° N, 47–49° W). There were 115–245 observations in each monthly average. The current speed varied from 2.5 to 7 cm per second, always in a southerly direction but tending slightly to the west of south from July to October and southeast the remainder of the year.

The log sheets from Station E usually contained estimates of ship drift. Examination of 73 records showed a mean current of 16 cm per second ESE. Individual observations varied widely in direction and speed, the latter ranging from 0 to 115 cm per second.

All such observations may be criticized on the grounds that it is virtually impossible to correct ship drift records for leeway. However, the maximum wind frequency in the weather ship observations was in the SW quadrant, while the maximum current frequency as well as total drift were SE. One would not expect the drift to fall so far to the right of the wind if leeway were a highly significant factor.

Four profiles of *Atlantis* hydrographic stations in the central Sargasso Sea (their positions will be shown later in Figure 4) provided additional data for the investigation of currents by the method of dynamic computation. The average surface current between the end stations of each profile ranged, in the different profiles, from 0.8 to 2.7 cm per second south, relative to the 1000-decibar surface, and 0.4 to 3.3 cm per second relative to 2000 db. There was a larger variation between adjacent stations, the total range being 13 cm per second north to 27 cm per second south. The currents were relatively uniform with respect to depth in the upper 300 to 500 m, and most of the shear occurred in the main thermocline between 500 and 1000 m.

Fuglister and Worthington (1951) have described the formation of eddies at the edge of the Gulf Stream, which transport water of temperate and Gulf Stream origin into the Sargasso Sea. The region is not as uniform and homogeneous as was formerly supposed. Occasional strong, southerly currents and rapid temperature changes at Station E suggest that it may be invaded by eddies from the north, and the travel time from the Gulf Stream might be as little as one to three weeks under exceptional circumstances. On the other hand, the average surface current

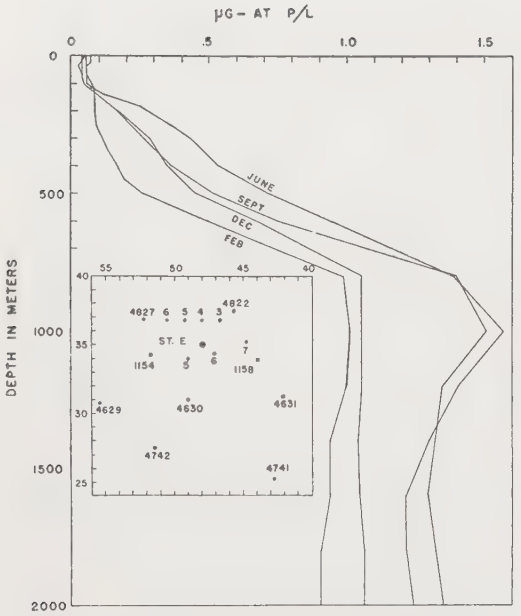

FIG. 4. Vertical distribution of phosphate in the central Sargasso Sea, determined by averaging groups of *Atlantis* stations. Their positions with reference to Station E are shown in the inset. Dates were as follows: 1144–8, Feb. 29 to Mar. 4, 1932; 4629–31, Dec. 21–24, 1947; 4741–2, June 6–9, 1948; 4822–7, Sept. 2–5, 1949.

was too feeble to suggest frequent or continuous invasion of significant proportions, and there is no reason to doubt that the water mass was essentially subtropical most of the time.

Chemical data

Figure 4 shows the mean vertical distribution of phosphate at stations in the central Sargasso Sea. The surface concentration was low and relatively uniform, and the December and February-March data showed no evidence of a winter maximum of the type found in temperate waters. Half of the stations had a minimum concentration at about 50 m, indicative of intense utilization at that depth. Below 100 m, the phosphate increased rapidly to a mid-depth maximum centered around 1000 m. Variations in mid-depths and deep water probably were due to differences in the relative amounts of Mediterranean and Antarctic Intermediate Water in the region at the time of sampling. The stations with the lowest mid-depth phosphate concentrations also had the highest salinity in the depth range of 1000 to 1500 m, where the Mediterranean influence is most pronounced.

The data show no evidence of seasonal change except possibly a slight summer increase in the seasonal thermocline between 100 and 500 m, followed by a reduction during the winter mixing period from December to March. Sampling variations were too large to estimate the magnitude of seasonal change or even to state positively that it exists. But the situation is obviously different from temperate waters, where destruction of the seasonal thermocline leads to an increase in the surface concentration. Hence if there was significant enrichment of the surface layer, there must have been a corresponding increase in the rate of utilization by phytoplankton.

Nitrate determinations have been recorded at a total of eight stations, in December, June, and July. Surface concentrations varied from 0 to 1.2 μg-at. N per liter, averaging 0.5 and again showing no evidence of seasonal variation. A mid-depth maximum of 20.4 to 24.4 μg-at. per liter was found at 1000 to 1200 m. Three surface nitrite values of 0.00 to 0.28 μg-at. N per liter have been recorded. The average concentration decreased with depth to 0.05 at 400 m.

Figure 5 shows a maximum oxygen concentration at the surface in February and at various depths from 40 to 100 m in other months. A mid-depth oxygen minimum of moderate intensity was located some 200–400 m above the phosphate and nitrate maxima. Further discussion is relegated to a later section in which these observations will be used in a quantitative analysis of rates of oxygen production and consumption in the upper 300 m.

PHYTOPLANKTON

The quantity of diatoms was highly variable, ranging from almost none to more than 100,000 cells per liter, but with the majority of samples within the range of one to three thousand. Dinoflagellates, coccolithophores, and silicoflagellates, listed in the order of their relative abundance, were less variable

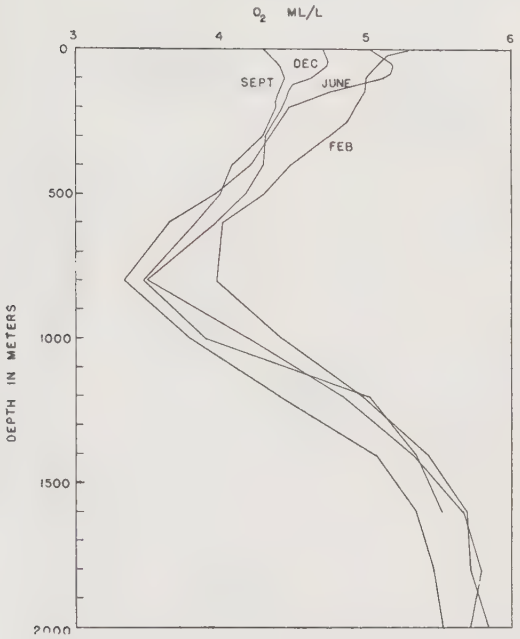

FIG. 5. Mean vertical distribution of oxygen in the central Sargasso Sea, determined from stations previously shown in Figure 4.

than diatoms, and the total of the three groups averaged about 1000 cells per liter. The most abundant segment of the population was the nannoplankton in the size range of 5 to 15 μ, which averaged about 100,000 cells per liter. This class contained a small number of identifiable organisms that would ordinarily be included in any routine count. There was a much larger number of cells that were indistinctive as to form and internal structure and were virtually unidentifiable in the preserved condition. In general, all such cells were counted, although it is unlikely that all of them were autotrophic organisms. Growing recognition of the importance of nannoplankton in the economy of the sea makes it desirable to include these small cells in the quantitative analysis of the population. The results must be viewed with reservations pending further taxonomic and physiological studies; however, later comparisons with pigment analyses will indicate that it is more accurate to include all unidentifiable cells than to omit all of them.

Even the larger forms present considerable difficulty to one who has not had an opportunity to examine large quantities of material. In most cases only a small number of any one species was available for study, and there was a large proportion of senescent and obviously atypical individuals. Hence it was decided that the material should be treated mainly from a quantitative point of view. Descriptions of species composition will be limited to cases where there is no reasonable doubt as to the correct identification and where the discussion will clarify ecological problems.

The seasonal cycle

Figure 6 shows the seasonal cycle of cell numbers, exclusive of nannoplankton, during the first year of the survey. The variable nature of the diatoms is clearly contrasted with the uniformity of the non-diatom population. There were small diatom pulses during the winter months, followed by a larger one in April, which developed within a week after the onset of thermal stability in the water column. It persisted in the surface layer for two weeks and could be traced into deeper water during the third week. Most of the midwinter species were present in the April augmentation. It was dominated by *Chaetoceros* spp. which had been present but not very important during the winter, and by *Rhizosolenia stolterfothii*, which had also been a dominant in the small February increase. Although the maximum observed concentration of cells was only 136,000 per liter, the sequence of events duplicated in miniature the vernal augmentation of temperate waters. Presumably the only essential difference was that the quantity of nutrients was insufficient to support a large production.

The diatoms declined in summer and autumn and generally were exceeded by dinoflagellates except for small, sporadic occurrences that tended to be localized both as to depth range and duration. The meaning of these patches will be discussed later. They contained some very large species and hence were more significant than is indicated in a graph of cell numbers.

The mean number of cells in the upper 150 m during the two-year period is illustrated in Figure 7A. Diatoms were plotted additively above the non-diatom fraction,

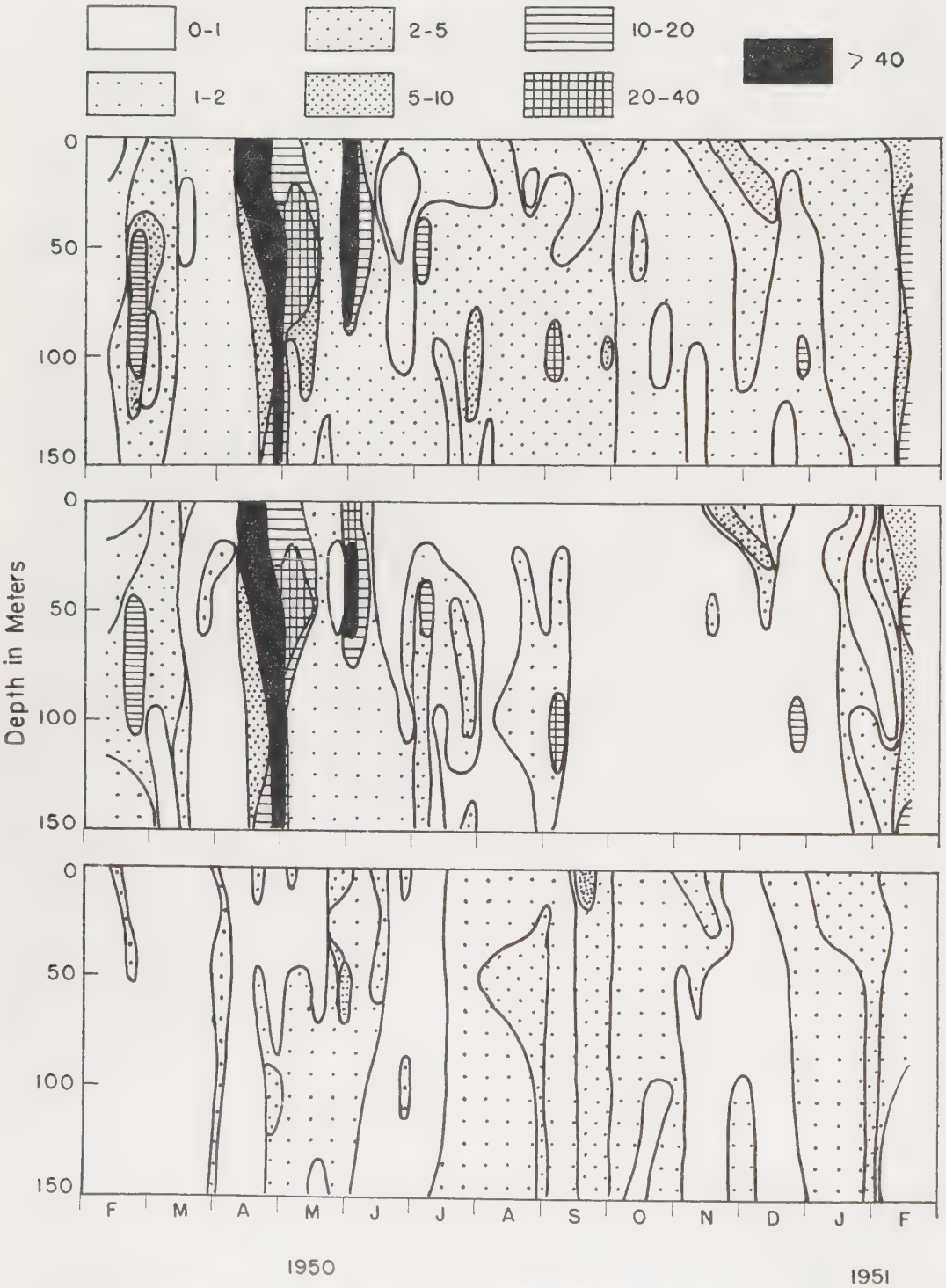

FIG. 6. Seasonal and depth distribution of phytoplankton as determined from cell counts. Symbols denote thousands of cells per liter. Upper figure, total cell count exclusive of small nannoplankton; middle, diatoms; lower figure, non-diatom fraction.

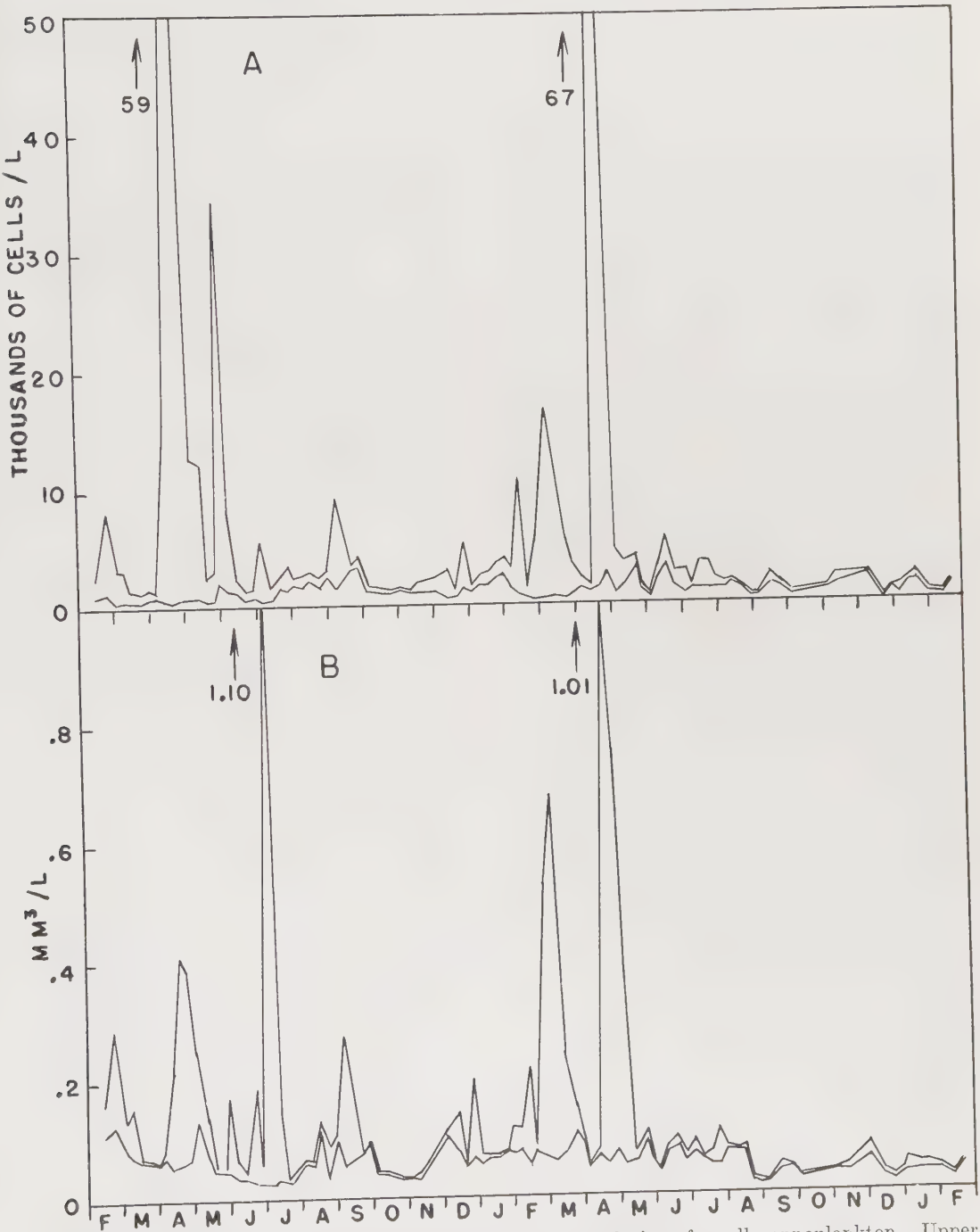

FIG. 7. *A*. Mean number of cells in the upper 150 m, exclusive of small nannoplankton. Upper curve, total cell count; lower, non-diatom fraction. Diatoms are indicated by the area between the two curves. *B*. Upper curve, total estimated phytoplankton volume; lower, nannoplankton and other non-diatom volumes.

so that the upper curve is the total number of cells in routine counts. In Figure 7B the nannoplankton volumes were plotted as the lower curve, the other fractions were added, and again the upper curve represents total phytoplankton.

In April, 1951, an increase of diatoms developed one week later than in 1950. The seasonal thermocline was not thoroughly established, but several incipient thermoclines appeared and were destroyed during the augmentation period. The species composition was similar to that of the previous year.

The data cover one winter period and parts of two others, and considerable winter variability is indicated. There was a February-March increase in 1951 with a volume of the order of magnitude of April growth. In the winter of 1951–2, the population remained at a low level, and in 1950 there was an intermediate condition.

Both years showed an irregular decline during the summer and early autumn, but there were minor differences between the two years. Diatoms were more abundant in 1950 than in 1951. In addition to the local patches previously mentioned, there was a more generalized increase in September, 1950.

Table 1 shows seasonal and depth variations in phytoplankton volume. Again it is apparent that there was little seasonal variation in the non-diatom fractions. The mean annual diatom volume exceeded the combined bulk of the remainder of the population. However, except in spring this was largely the statistical result of the sporadic occurrence of relatively large diatom floras. In a majority of individual samples the nannoplankton dominated the other groups.

Seasonal averages of the vertical distribution of diatoms showed an increase from the surface downward to a maximum at 50 or 100 m. The reduction in the surface population was particularly marked in summer and autumn, suggesting a rapid sinking rate. The non-diatom population had a maximum at the surface in autumn and winter. During other seasons there was a tendency toward double maxima at 0 and 50 m. Most of these forms were motile, and the observed distribution probably indicates their preferred depth. The maximum quantity of nannoplankton occurred at 0 and 25 m, but appreciable amounts were found in the deepest water sampled. These small cells must sink slowly if at all, and their maintenance in substantial numbers below 100 m suggests an ability to grow actively on organic carbon sources. In all fractions of the population there were enough cells at 150 m to indicate that deeper sampling would be required to capture all of the phytoplankton. Riley (1939) found a measurable concentration of plant pigments at 400 m, and the concentration at 200 m was nearly as large as that of the surface layer. On the other hand, Lohmann (1920) and Riley and Gorgy (1948) found little plankton below the present sampling depth. Probably the latter is fairly adequate except during the decay of a plankton pulse.

TABLE 1. *Seasonal averages of estimated phytoplankton volume in mm³ per liter*

Depth (m)		1950–51						1951–52
		0	25	50	100	150	Mean	Mean
Winter, Dec. to Mar.	Nannoplankton	.093	.099	.050	.062	.054	.066	.045
	Diatoms	.015	.020	.037	.097	.031	.051	.089
	Other	.008	.005	.003	.009	.005	.007	.004
	Total	.116	.124	.090	.168	.090	.124	.138
Spring, April and May	Nannoplankton	.099	.083	.056	.049	.050	.062	.053
	Diatoms	.123	.195	.244	.056	.058	.137	.252
	Other	.003	.002	.005	.007	.009	.004	.007
	Total	.225	.280	.305	.112	.117	.203	.312
Summer, June to Aug.	Nannoplankton	.043	.047	.039	.034	.036	.039	.048
	Diatoms	.003	.028	.244	.074	.199	.124	.013
	Other	.008	.006	.008	.005	.007	.007	.006
	Total	.054	.081	.291	.113	.242	.170	.067
Autumn, Sept. to Nov.	Nannoplankton	.079	.060	.037	.037	.032	.044	.025
	Diatoms	.013	.007	.011	.066	.004	.028	.004
	Other	.010	.004	.008	.007	.001	.006	.005
	Total	.102	.071	.056	.110	.037	.078	.034
Annual, Mean	Nannoplankton	.078	.074	.045	.047	.043	.053	.044
	Diatoms	.029	.048	.117	.077	.071	.078	.083
	Other	.008	.004	.006	.007	.006	.006	.005
	Total	.115	.126	.168	.131	.120	.137	.132

ECOLOGICAL DISCUSSION

Species composition in relation to water movements

Table 2 lists dominant diatoms, arbitrarily defined here as species which constituted 5% or more of the total population on any one sampling date. Many of the species are subtropical or cosmopolitan, but the list also includes temperate and even neritic species. This indicates in some cases that our concepts of species habit need to be broadened. For example *Dactyliosolen mediterraneus*, which Cupp (1943) described as "neritic, sporadically oceanic," was found throughout the year although dominant only in winter, and it certainly should be regarded as a resident species. *Ditylium brightwellii*, another species commonly regarded as neritic, was found sporadically throughout the summer and undoubtedly has oceanic tendencies. All of the species listed were present on a number of occasions and presumably were capable of growing successfully in the region except possibly some of the last five in the summer and autumn group. They are the species that comprised the localized patches found in the seasonal thermocline in summer, as previously shown in Figure 6. Four of the five are regarded as being temperate water diatoms, either oceanic or neritic. Their occurrence in large concentrations, often at a single depth on a particular sampling date, suggests that they may have been transferred into the region from the north in small-scale lateral eddies. The water temperature in the patches ranged from 17 to 20°. There would be no density barriers to hinder the movement of temperate surface water southward and downward to a subsurface position at Station E.

There also exists the possibility of water movements of larger scope and more profound biological influence. The physical oceanographic description indicated that the region is sometimes invaded by water masses from the north which constitute a general movement of the surface layer rather than limited penetrations along density surfaces. Paradoxically, the more important such movements are, the more difficult it is likely to be to recognize them. The biological result of the invasion of a water mass more than 150 m thick during two or more collection dates may be virtually indistinguishable from a change in the population due to local ecological conditions if the species concerned are cosmopolitan. There was a large increase in the total cell count on May 31, 1950, and a gradual return to normal during the next two weeks. The species composition changed, but it included both temperate and subtropical forms. Neither the phytoplankton nor the temperature records give a clear indication of what happened.

The increase of September, 1950, was dominated by *Rhizosolenia hebetata* f. *semispina*, which also is sometimes an important component of autumn augmentations in temperate waters. The species was present

TABLE 2. *List of dominant diatoms (species constituting 5% or more of the total cell count on any one sampling data)*

With one exception (*Ditylium brightwellii*) the nomenclature follows Cupp (1943)

Winter	Summer and Autumn
Rhizosolenia stolterfothii	*Chaetoceros* spp.
R. alata	*Corethron hystrix*
* *R. hebetata* f. *semispina*	*Bacteriastrum delicatulum*
Bacteriastrum delicatulum	*Thalassiothrix mediterranea*
B. elongatum	*T. frauenfeldii*
Chaetoceros spp.	*Stephanopyxis palmeriana*
Nitzschia sp.	*Rhizosolenia calcar-avis*
Schröderella delicatula	*R. hebetata* f. *semispina*
Hemiaulis hauckii	* *R. styliformis*
Dactyliosolen mediterraneus	* *R. cylindricus*
Corethron hystrix	* *Coscinodiscus centralis*
Stephanopyxis turris	* *C. concinnus*
Thalassiosira frauenfeldii	* *Ditylium brightwellii*

Spring Flowering	
Chaetoceros messanensis	*Rhizosolenia stolterfothii*
C. peruvianus	*R. alata*
C. laciniosus(?)	*Nitzschia* sp.
C. lorenzianus(?)	
C. brevis(?)	

* Localized concentrations, possibly in eddies of temperate water origin.

for three weeks in widely varying amounts in the various samples, and it had a maximum concentration of 21,000 cells per liter at 100 m on September 7. Here again there is no certain evidence as to whether local production was observed, or an invasion by a population from the north.

Ecology of the seasonal cycle

The review of chemical data indicated that the concentrations of phosphate and nitrate in the surface layer were low and relatively constant throughout the year. Average concentrations of 0.05 μg-at. P per liter and 0.5 μg-at. N must have a strongly limiting effect on the growth rate, and they are insufficient to support production of temperate water proportions. Application of average plankton conversion factors indicates that the mean population of 0.13 mm^3 contained about 0.005 to 0.015 μg-at. P per liter in organic combination. Utilization of all available inorganic phosphorus would result in a crop about three to ten times the average population. Observed increases fell within these limits.

Nevertheless, the rapid sequence of little pulses during two of the three winters indicated a rapid rate of nutrient renewal during the season of overturn. Vertical mixing in the upper few hundred meters was the most likely source of enrichment. Earlier studies of deep water diffusion by Riley (1951a) indicated that only a few per cent of the annual nutrient requirement is derived from depths greater than 300–500 m.

The winter population at Station E differed markedly from that of temperate waters, where a pronounced minimum of two months or more ordinarily precedes the spring increase. Nutrients are generally abundant in temperate waters at that time, so that the midwinter minimum is usually ascribed to low light intensity and turbulence. These factors are presumably less severe at Station E, but a simple analysis will show them to be suboptimal.

Assume that the population in a water column of z meters' depth is subjected to strong turbulence, so that the amount of light received by any one cell approaches the mean amount of light $\bar{I}$ in the water column as a whole. This is given by

$$\bar{I} = \frac{I_0(1 - e^{-Kz})}{Kz}$$

where I_0 is the incident light, and K is the extinction coefficient. A generalized estimate of the amount of light available in temperate waters as calculated from this equation is listed in Table 3. The use of total incident radiation rather than the wave lengths used in photosynthesis is permissible since the purpose is only to obtain a regional comparison rather than to estimate the absolute amount of light.

In the shallow waters of Cape Cod Bay, a major augmentation has appeared as early as mid-December (Bigelow 1926). In Long Island Sound, with a mean depth of 20 m, January and February increases have been observed by Riley (1956). In depths of 30 m, maxima have been reported in late January in the coastal water south of Woods Hole (Lillick 1937) and in late February in Block Island Sound (Riley 1952). The vernal augmentation begins in March in waters of 30 to 55 m on Georges Bank (Riley 1941). In all of these cases a mean radiation of about 0.03 g-cal cm^{-2} min^{-1} appears to be critical for the initiation of active production. By the same criterion, April and May pulses would not be possible in turbulent waters of greater depth than 60 or 70 m. Therefore, it is consistent with the general theory that April and May flowerings coincided with the formation of the seasonal thermocline in the

TABLE 3. *Mean amount of light (g-cal cm^{-2} min^{-1}) in a water column with values for depth (z) and incident ration (I_0) as listed, and with an extinction coefficient of 0.15*

Depth (z)	Dec.	Jan.	Feb.	Mar.	Apr.	May
0 ($=I_0$)	.086	.094	.138	.212	.272	.306
10	.045	.049	.072	.110	.140	.158
20	.027	.030	.044	.067	.086	.097
30	.019	.021	.030	.047	.060	.067
40	.014	.016	.023	.036	.045	.051
50		.013	.018	.028	.036	.041
60			.015	.024	.030	.034
80				.018	.023	.025
100					.018	.021

TABLE 4. *Mean amount of light (g-cal cm^{-2} min^{-1}) in a water column with values for depth (z) and incident radiation (I_0) as listed, and with an extinction coefficient of 0.075*

Depth (z)	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
0 (=I_0)	.148	.117	.124	.154	.230	.279
40	.047	.037	.039	.049	.073	.088
50	.039	.031	.032	.040	.060	.073
60	.033	.026	.027	.034	.051	.061
80	.025	.019	.021	.026	.038	.047
100	.019	.015	.016	.020	.030	.036

Gulf of Maine (Bigelow, Lillick, and Sears 1940), the deeper parts of Georges Bank, and the slope water south of the Bank (Riley 1941).

Table 4 shows a similar calculation for Station E. In November the depth of the mixed layer was about 60 m. By temperate water criteria, active growth was possible, nutrient conditions permitting. The mixed layer gradually deepened between December and March to 150 m or more, and the complex of light and turbulence became critical. A plankton pulse would be impossible if turbulence were strong enough to distribute the plankton uniformly through the mixed layer. However, the existence of measurable thermal gradients in a considerable proportion of the midwinter bathythermograms is sufficient evidence that the water column was not thoroughly mixed at all times. In such cases, an increase should be possible in the upper 50 m.

Figure 8 compares winter phytoplankton volumes with daily averages of the vertical temperature gradient in the mixed layer. Zero and positive gradients suggest strong mixing, and negative gradients are indicative of at least a slight degree of stability in the water column. Comparing one year with another, there was clearly a relationship between the frequency of negative gradients and the development of a large winter population. In following the sequence of events through any one winter season, a similar relationship could be observed in some cases, but it was not consistent. In this respect it is unfortunate that the large increase of March, 1951, occurred at a time when no bathythermograms were taken. It would be interesting also to know whether the sparse population of the winter of 1951–2 was followed by an unusually large spring production due to the accumulation of unused nutrients in the surface layer.

Figure 8 shows that the spring augmentation of 1950 accompanied the formation of the seasonal thermocline. In 1951 there was an increase in the magnitude of negative gradients at the time of most active growth, but the latter was terminated before the thermocline was fully established. The earlier analogy with spring growth in temperate water still seems justified, although the superior light intensity at Station E reduced the requirement for strong stability. However, it is also apparent that there was no distinct ecological difference between the April augmentation and those which occurred during the winter. The same species were present, although *Chaetoceros* was relatively more important in spring, and the ecological requirements for initiation of active production were also similar. The chief difference was simply a matter of predictability. Winter pulses, depending on vagaries of the weather, were sporadic and generally doomed to quick extinction. The vernal increase in illumination and stability tended to insure a spring increase which would run its full course at nearly the same time every year.

The seasonal thermocline gradually became stronger through the spring and summer. In early autumn the mixed layer began to deepen gradually, but serious destruction of the thermocline did not begin until December. The summer period of minimum nutrient replenishment therefore may be regarded as lasting into late autumn. The population was very sparse in the upper 25 m through all of this period, and the total population also declined to an autumn minimum. Irregular variations at 50 to 150 m have been discussed in connection with temperate water eddies, but it is also possible that local growth was more effective in the upper part of the thermocline than in the immediate surface layer. Several species of *Peridinium*, *Ceratium*, and *Dinophysis* appeared to be thriving at depths of 50 or

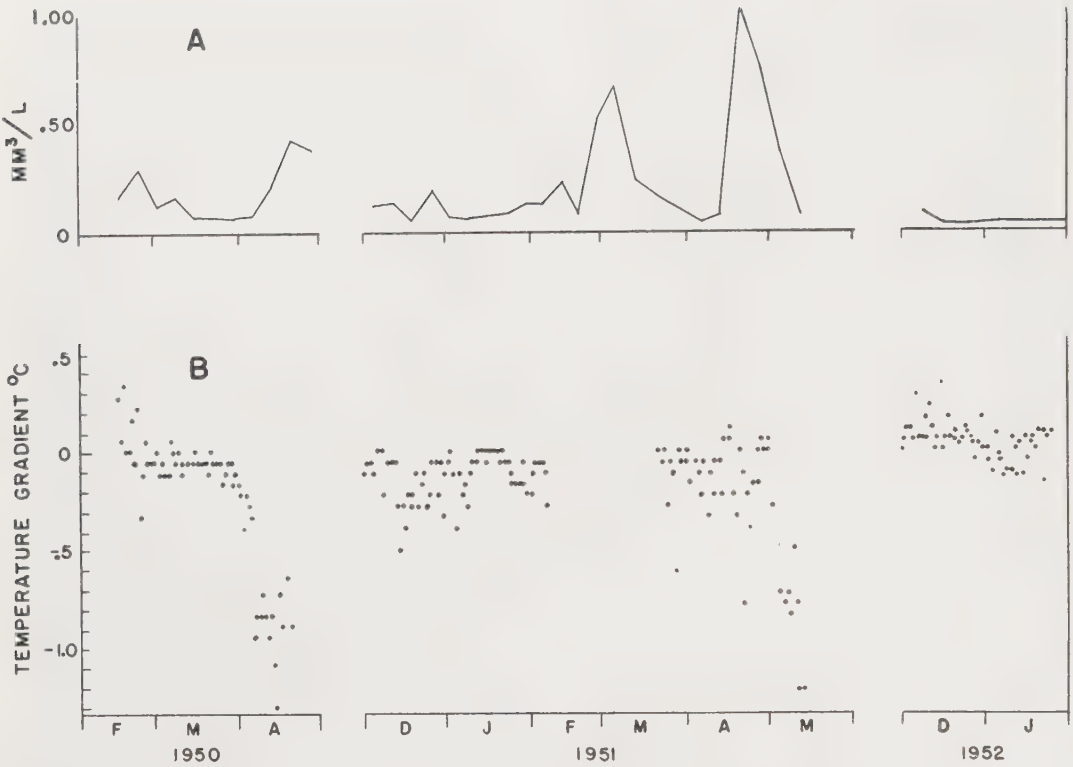

FIG. 8. *A*. Total estimated phytoplankton volume in winter. *B*. Daily record of the average difference in temperature between the lower part of the mixed layer and the surface.

100 m, where presumably the decrease in light intensity with depth tended to be compensated by an increasing nutrient concentration.

The zooplankton population described by Fish (1954) showed a remarkable degree of agreement with the phytoplankton. The zooplankton had a spring maximum and an early winter minimum one to two months after the corresponding features of the phytoplankton cycle. Variations in winter zooplankton volumes from one year to the next were also similar, the largest populations occurring in the late winter of 1951, the smallest in 1952.

Comparison with earlier work

Previous cell counts consisted of a series of stations in the eastern Sargasso Sea described by Lohmann (1920), in which there was an average of 80 million cells in the water column below 0.1 m^2 of sea surface between 30° and 40° N and 24 million between 20° and 30° N. In the present work, the April-May average of 344 million cells per column exceeded Lohmann's largest values, but averages for other seasons ranged from 44 million in autumn to 61 million in winter.

Riley, Stommel, and Bumpus (1949) have summarized phytoplankton pigment analyses in the Sargasso Sea. The extremes ranged from 175 Harvey units per m^3 in July, 1947, to 2100 in December of the same year. Both figures are cruise averages for the upper 200 m. The relationship between pigment units and other phytoplankton measurements has not been examined carefully in the Sargasso Sea, but an order-of-magnitude estimate may be obtained by applying conversion factors determined elsewhere. In Long Island Sound, Harris and Riley (1956) found that chlorophyll *a* averaged 0.33% of the dry weight of phytoplankton and 0.043% of the wet weight. One μg of chlorophyll was equivalent to 4.4 Harvey units. On this basis, the range of pigment

analyses cited above would be equivalent to 0.08 to 1 mg wet weight, or approximately the same units in mm^3. This includes most of the range of cell volumes obtained during the present survey, provided the so-called nannoplankton fraction is included in the volume estimates. The quantity of material in routine cell counts, excluding the nannoplankton fraction, frequently was insufficient to account for more than a small fraction of the minimum observed plant pigments.

Phytoplankton productivity and animal food requirements

The general level of production and consumption in the Sargasso Sea has been and remains in dispute, largely because of errors in experimental methods and questions as to their correct interpretation (Steemann Nielsen 1952, 1954, Riley 1953, Ryther 1956). In reopening the problem at the present time it is therefore necessary to develop independent methods of analysis that are free from previous objections.

The first method to be examined is the calculation of biological rates of change of oxygen by physical oceanographic methods. The bathythermograms at Station E provided a detailed picture of seasonal temperature changes, from which it was possible, during most of the year, to calculate coefficients of vertical eddy conductivity. The methods used were a more elaborate version of the technique used by Riley and Gorgy (1948) in an earlier study of summer production rates in the Sargasso Sea. The usual differential equation for the local time rate of change of temperature may be written for purposes of numerical computation in the finite difference form,

$$\frac{\partial T}{\partial t} = \frac{A_a(T_1 - T_2) - A_b(T_2 - T)_3}{z^2},$$

where T_1, T_2, etc. are the temperatures at successive depth levels z distance apart, and A_a, etc. are average coefficients of vertical eddy conductivity for each interval. The average rate of change of temperature by monthly intervals and the average vertical gradients were determined from the data used in drawing Figure 3B. Substitution of numerical values for temperature led to a series of equations for successive depth levels, each containing two unknown values of A. Solution of the series depends on the fact that the eddy coefficient is small and relatively constant below the depth of seasonal temperature variation. Therefore, hydrographic data were used to extend the bathythermograph data to a depth of 300 m, at which point A_a was assigned a value of 0.6, in accordance with an estimate obtained by Riley (1951a). This permitted a solution for the value of A_b. The equation for the next level upward, containing A_b and A_c, could then be solved, and so on, successively, to the surface. It should be noted in passing that the deep water values could have been altered through a liberal range of about 0 to 1 without altering the larger coefficients near the surface more than a few percent.

The calculation was carried out for the whole year, but it was found that in winter the thermal gradients in the mixed layer were too small and variable for practical application of the method. Therefore, a theoretical estimate was obtained from a formula given by Riley (1951b) for water of indifferent stability, which depends upon the mean current velocity and the depth of the mixed layer. Values so obtained were too small to produce the observed amount of cooling with the gradients that were actually present in the water, but this is not a serious defect because there probably was considerable cooling by convection.

The eddy coefficients were then applied to the available oxygen data for the area, which was previously illustrated in Figure 5, in order to determine the biological rate of production and consumption at a series of depths. Numerical values for the coefficients are shown in Table 5, averaged for the depth ranges required. The method of analysis has been described in detail by Riley and Gorgy (1948). The principle is simply that a certain part of the observed change in oxygen at a given depth is due to vertical diffusion, and it is assumed that this may be determined from the observed oxygen gradient and values for A computed

TABLE 5. *Calculated coefficients of eddy conductivity used in the computation of net rates of change of oxygen in the upper 300 m*

Depth (m)	cm²/sec		
	2–5 Sept.	21–24 Dec.	6–9 June
0–50	5.7	30	2.4
50–100	4.8	25	1.9
100–150	4.8	3	2.1
150–200	4.8	3	0.6
200+	0.6	0.6	0.6

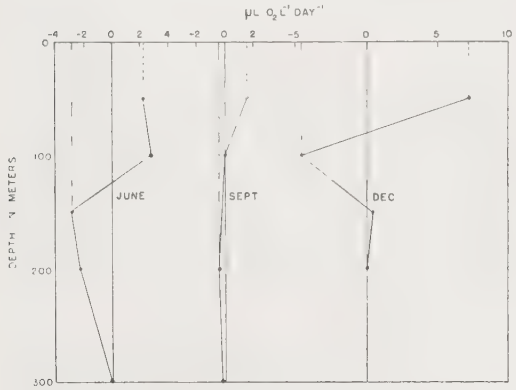

FIG. 9. Biological rate of change of oxygen in the upper 300 m, estimated from physical oceanographic calculations of oxygen production and consumption. See text for further details.

from the distribution of a conservative property. Any imbalance that remains is then due to biological processes *in situ*.

The February-March data shown in Figure 5 were omitted from the calculation for two reasons. The great depth of the mixed layer at that time made the determination of eddy coefficients very doubtful. In the second place, the curve in Figure 5 suggests that production was mainly confined to the upper 50 m or less, and more depths would have to be sampled in order to give usable results.

Unfortunately the oxygen data were not as abundant nor as detailed as might be desired, nor were the observations obtained during the same year as the temperature observations. Nevertheless, Figure 9 presents a realistic and fairly consistent picture. Positive values in the upper 85 to 125 m indicate phytoplankton production in excess of the respiratory requirements of the whole biological association and hence are a minimum estimate of net phytoplankton production. Better results would have been obtained if the oxygen observations had been spaced more closely near the surface, but a rough estimate of the total may be made by assuming that production was uniform in the upper 50 m, as indicated by the dotted lines in the figure. Between the compensation point and 300 m, the area to the left of the zero line may be used similarly for an estimate of consumption in excess of photosynthesis. If one were to assume that consumption was uniform from the surface to the depth of maximum observed consumption, as indicated by the dashed lines, then the total area to the right of this line would represent total photosynthesis. Such estimates will be made but are obviously of a lower order of validity.

Before summing the results, it is desirable to consider the problem of deep water consumption, illustrated in Figure 10. Riley (1951a) made an analysis of the biological rate of change of oxygen and phosphate in mid-depths and deep water of the Atlantic Ocean, again by standard methods of physical oceanographic analysis. Figure 10 shows the calculated mean oxygen consumption in the latitude range of 18° to 27° N. An estimate of animal respiration in deep water is shown as a superimposed dotted line. This is based on tows by Leavitt (1935, 1938) in the Sargasso Sea and on the experimentally observed oxygen consump-

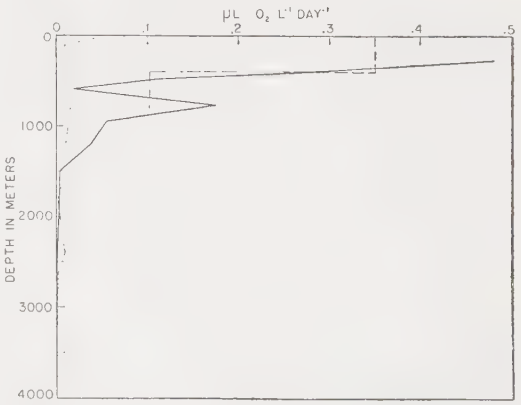

FIG. 10. Oxygen consumption in deep water, based on physical oceanographic calculations (solid line) and estimates of zooplankton respiration (dotted and broken lines).

tion of surface zooplankton populations in winter, described by Riley, Stommel, and Bumpus (1949). The latter authors also obtained measurements of zooplankton volume from tows with Clarke-Bumpus samplers in the upper 800 m, which, together with experiments on zooplankton respiration made concurrently, provided an estimate of animal respiration in the surface layer and upper mid-depths, which is shown as a broken line. The Clarke-Bumpus tows obtained small organisms which escaped the coarser nets used by Leavitt, and hence they provided a more reliable estimate of total respiration in near-surface waters. The general concordance of the results obtained by independent methods—experiments and physical oceanographic calculations—suggests that they are at least of the right order of magnitude.

The data shown in Figures 9 and 10 were converted to estimates of carbon production and consumption and the results are summarized in Table 6. The table also includes an estimate of net production for the period of July to September, 1947, which was published by Riley and Gorgy (1948) but was not included in Figure 9. With the help of the seasonal cycle of phytoplankton as determined by cell counts, a crude estimate of the annual mean production may be made. Cell counts remained relatively low from mid-June until early December, so that the low summer values for net production appear to be applicable to about a six-month period. The relatively high values obtained on December 21–24, 1947, accompanied a flowering that was general throughout the Sargasso Sea. Although the cell counts at Station E showed occasional major increases throughout the winter and spring, the total duration of high production rates probably was not much more than a month. The intermediate June data were applied to the remaining five months of the winter-spring period. This method of calculation was applied to the net increase in the surface layer, the gross production, and the net decrease between the compensation point and 300 m. To the latter was added another 0.06 g C per m², which is the sum of the deep water consumption as indicated by physical oceanographic analyses in Figure 10. Good agreement was obtained between the excess production above the compensation point and the excess consumption below that point.

TABLE 6. *Daily rate of change of carbon in g/m² of areal surface computed by physical oceanographic methods from the vertical distribution of temperature and oxygen, also animal respiration calculated from quantitative collections and experimental measurements of oxygen consumption*

Atlantis stations	4606–17	4629–31	4741–2	4822–7	
Dates	26 July–4 Sept. 1947	21–24 Dec. 1947	6–9 June 1948	2–5 Sept. 1949	Est. Ann. Mean
Net increase, surf. to CP	.05	.49	.15	.06	.134
Gross production	—	.89	.33	.09	.272
Net decrease, CP-300 m	—	.15	.14	.03	.081
Net decrease, CP-bottom	—	.21	.20	.09	.143
Animal respiration	.12	.04	.16	—	.106

CP = Compensation point.

The experimental measurements of zooplankton respiration and quantitative measurements of the population were used to calculate the total consumption by zooplankton from surface to bottom. The results are shown in the last line of Table 10. The estimated annual mean is simply the arithmetic mean of the available data, and again there is fair agreement with the other estimates.

It is apparent from the methods of calculation that each estimate, for one reason or another, falls short of the full value for net phytoplankton production. In the case of the net increase above the compensation point, it would be necessary to add the consumption by zooplankton and bacteria in the same depth range. The former probably amounts to about 0.02 g C per m²; the latter has not been estimated. In round numbers, the daily net phytoplankton production is probably at least 0.15 g, or 50 g C per m² as an annual total. By way of comparison with productive temperate waters, Riley (1956) estimated an annual total of 150 g for Georges Bank and 205 g for Long Island Sound.

In two of the three groups of data that

have been used in the oxygen analysis, there were sufficient phosphate observations for a determination by similar methods of the net rate of utilization of phosphate, again using the eddy coefficients listed in Table 5. The 50 m level in the December data yielded a net rate of 2.7×10^{-3} μg-at. P L^{-1} day^{-1}. In September, 1949, the rate was 7.3×10^{-4} μg-at. P at 50 m and 3.9×10^{-4} at 100 m. Below these depths the rates were positive, indicating an excess of regeneration over utilization. However, numerical values for net regeneration rates cannot be given with any degree of confidence. It was previously pointed out in connection with the vertical phosphate distributions in Figure 4 that it cannot be decided with certainty whether there was significant accumulation of phosphate in the seasonal thermocline. A better knowledge of these possible seasonal changes would be necessary for accurate determination of the biological rate of change.

Below the depth of seasonal temperature changes, the average phosphate gradient may be used to determine the rate of upward transfer from deep water. Assuming an eddy coefficient of 0.6 cm^2/sec, the vertical flux is found to be 5×10^{-4} μg-at. P per cm^2 of horizontal surface at the 300 m level. This probably is of the order of one-tenth the minimum daily requirement as indicated by the calculated net rate of consumption in September, 1949. Thus both oxygen and phosphate data point toward the conclusion that the major biological activity of the system, consumption as well as production, occurred in the upper 300 m.

One further comparison should be made. At the 50 m level in the September data, the ratio of estimated carbon production to phosphate utilization was 92 atoms of carbon to one of phosphorus. In December the ratio was 109:1. Sverdrup *et al.* (1942) reported that the average C:P ratio in plankton is 106:1. Realistic atomic ratios support the conclusion that vertical gradients of oxygen and phosphate can be determined with sufficient accuracy to warrant the kind of analysis that has been presented. However, further thought needs to be given to methods of computing eddy coefficients and converting net rates of change to estimates of total production and consumption.

Previous studies of the Sargasso Sea have yielded a variety of results, which should be discussed briefly. Steemann Nielsen (1954) listed three C^{14} experiments in the central Sargasso Sea which were obtained in June, 1953, and averaged 0.05 g C per m^2. He regarded this figure as representing gross production and assumed that it was suitable for estimating an annual mean. Neither conclusion seems likely. The seasonal variation in standing crop and production rates described herein are sufficient evidence that a set of observations taken during any one month cannot be inflated to the status of an annual mean. Also, according to Ryther (1956) the C^{14} method essentially measures net production rather than total photosynthesis. Numerically Steemann Nielsen's figure is less than one-fifth of the mean gross production in Table 6 but is equal to the minimum value for net production obtained during the period from July to September, 1947.

Riley (1953) reported that a daily surface production of 0.3 g C per m^2 was required to feed the zooplankton in the northwestern Sargasso Sea. This calculation was based on an extensive series of semi-quantitative net hauls in the area northwest of Bermuda, described by Clarke (1940). The two- or three-fold difference between that estimate and the present one cannot be rationalized at this time. It may represent a geographical variation in different parts of the Sargasso Sea, or there may be sampling errors involved or errors in converting Clarke's semi-quantitative data into a quantitative estimate.

Worthington (1954) reported that the oxygen content of the deep water mass below 2000 m in the central Atlantic has decreased about 0.3 ml per liter during the past 20 years and postulated that this was due to biological consumption. Converted to the units used here, it is equivalent to a daily utilization of about 0.05 g C per m^2, some 30 times the present estimate of consumption in the same depth range and one-third of the total surface production. If Worthing-

ton is correct, the estimates of surface production are probably much too low, for even the shallow, coastal bottom fauna is seldom able to acquire such a large fraction of surface production. Worthington's conclusion is open to question until he proves that the observed decrease in oxygen is due to consumption *in situ* rather than infusion of water of lower oxygen content from southern latitudes. However, in either case he has cast doubt upon the validity of present estimates of total oxygen consumption in deep water, since these assumed a steady state condition.

In short, the present work has yielded three consistent sets of values for net production and consumption, which fall well within the range of previous estimates. While mere consistency of the results of independent methods does not prove their validity, they provide a more realistic approach than the piecemeal investigations of the past.

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The Determination of Calcium in Natural Waters[1]

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ABSTRACT

A procedure for the determination of calcium in natural waters is presented which is faster, more precise, and more accurate than those previously described. Photometric weight titration with ethylenediamine-tetraacetate ion in the presence of ammonium purpurate is employed following isolation of the calcium by ion-exchange chromatography. Calcium is separated from magnesium and strontium by the use of acetylacetone as a complexing elutant.

Samples of sea water from the Bahamas region were examined by this procedure, and good agreement was found with reported values determined by the classical triple oxalate procedure. The calcium content is one per cent higher than that which would be expected from the accepted calcium-chlorinity ratio. In addition, samples from the Chesapeake Bay indicate that the sea water source for this region has a greater calcium content than would be expected from the accepted calcium-chlorinity ratio for sea water.

INTRODUCTION

The methods for determining calcium which have been reported in the literature include nearly all the common analytical techniques. The calcium in sea water has been most frequently estimated by oxalate precipitation and the precipitate either ignited to the oxide and weighed (Thompson and Wright 1930, Matida 1950) or dissolved in sulfuric acid and the oxalic acid titrated with potassium permanganate (Kirk and Moberg 1933, Gripenberg 1937). These procedures have in common the difficulties encountered in quantitative precipitation with oxalate in the presence of magnesium and strontium. The ratio of magnesium to calcium in sea water is approximately 3:1 on a weight basis and 5:1 on an equivalent basis. The ratio of strontium to calcium in sea water is approximately 0.03:1 on a weight basis and 0.015:1 on an equivalent basis. Examination of a few of the careful studies on oxalate precipitation shows that this composition makes separation very difficult (Kolthoff and Sandell 1943, Ingols and Murray 1949, Holth 1949, Gordon and Wroczynski 1952). There is marked disagreement among the recommended procedures. Compensating errors arise from postprecipitation of magnesium and incomplete precipitation of calcium as temperature conditions and digestion periods are varied. Sodium, potassium, and sulfate have an effect on the composition of the precipitate. All of the methods show some coprecipitation with equal amounts of calcium and magnesium, so that multiple precipitation is necessary. Errors arising from these extensive manipulations and solubility losses are difficult and tedious to avoid. Strontium is supposedly precipitated quantitatively with the calcium but the opposite opinion has been expressed (Banewicz and Kenner 1952, Chow and Thompson 1955b). Webb (1938) has proposed a correction for the strontium included in sea water calcium analyses. The oxalate procedures for sea water are laborious and not susceptible to rigorous interpretation.

A flame photometric procedure for calcium in sea water has been described (Chow and Thompson 1955a). Chloride, magnesium, potassium, and sulfate ions showed a negative radiation interference, and sodium

[1] Portions of this work received financial support from funds furnished by the U. S. Public Health Service to The Johns Hopkins University under research grant number RG 4430, and funds furnished to the Chesapeake Bay Institute of The Johns Hopkins University by The Office of Naval Research, the State of Maryland (Department of Research and Education), and the Commonwealth of Virginia (Virginia Fisheries Laboratory). (Contribution Number 32, The Chesapeake Bay Institute.)

ions gave a positive radiation interference. When the method of standard additions was used to compensate for these interferences, a precision of one per cent was obtained.

When the analyses of open ocean water by various investigators are compared on the basis of calcium to chlorinity ratios, agreement to within one per cent is found (Dittmar 1884, Thompson and Wright 1930, Kirk and Moberg 1933, Gripenberg 1937, Matida 1950, Chow and Thompson 1955a). This constancy of composition will allow calcium estimation from the chlorinity with nearly as great precision as the direct determination. In samples that have a greatly altered calcium-chlorinity ratio, the possible change in relative concentration of the other ions makes the use of the empirical gravimetric procedures questionable.

The determination of calcium in natural waters other than sea water may have the added difficulties that quite dilute solutions must be analyzed, and appreciable quantities of metallic ions other than members of Periodic Groups Ia and IIa may be present.

THE DETERMINATION OF CALCIUM IN PURE AQUEOUS SOLUTIONS

The estimation of calcium in a solution which does not contain other cations is most readily performed by complexometric titration. The large number of papers in the literature on the use of ethylene-diamine tetraacetate ion (EDTA) for titration of calcium ion supports this suggestion. The determination of calcium in natural waters with EDTA has been described (Betz and Noll 1950). Perhaps the most important feature of complexometric titrimetry, which has contributed to its popularity, is its speed. However, in addition, complexometric titration may be refined, so that an exceedingly accurate, precise, and sensitive analytical procedure may be proposed. A few milligrams of calcium may be estimated to fractions of a microgram.

Theoretical

Titrimetry is based on the discrimination of the point at which an amount of reagent equivalent to the constituent has been added. The sensitivity of the equivalence point detection and the formation constant for the reaction are the parameters which determine the obtainable accuracy and precision. As the equivalence point is approached, the reverse reaction may cause deviation from a linear (stoichiometrical) relationship between added reagent and concentration of the constituent being titrated. The use of a supposed linear relationship between added reagent and concentration of active constituent has the advantages that the information from several measurements is combined so that the effect of random variables is decreased, in contrast to logarithmic relationship employed in potentiometric titrations. The establishment of this relationship is easier than the addition of exactly the correct amount of titrant, which implies that the final addition is infinitely small. The precision with which this relationship can be determined is a function of the sensitivity of the procedure used to follow the course of the titration.

Spectrophotometric estimation of the concentration of one of the components of the system is a very sensitive means of determining the progress of the titration. The spectrophotometric titration of calcium with EDTA has been studied (Sweetser and Bricker 1954). Solutions as dilute as 0.00003 molar were titrated with sharp end points. Ultraviolet absorption at 222 mμ was used to determine the excess EDTA. The EDTA was found to have an absorbancy index of 10^3/mole/liter/cm at this wave length. The volumetric methods used appear to limit the precision to one part per thousand. Solutions containing magnesium were titrated in the presence of 0.6 molar sodium hydroxide and 0.016 molar citrate, but coprecipitation of calcium with equal amount of magnesium was one per cent and large blanks were found. A severe restriction on this system is the use of the far ultraviolet where many compounds have strong absorption.

A photometric procedure for calcium using ammonium purpurate as a color developing agent has been examined (Williams and Moser 1953) and has a high sensitivity. The absorbancy index of the calcium purpurate complex is 10^4/mole/liter/cm. At

pH 11.3 and a purpurate concentration of 5×10^{-5} mole/liter, Beer's law was found to hold up to 1.2 ppm calcium. The procedure may be adapted to photometric titration. The use of this rather unsatisfactory visual indicator is not only more sensitive than the ultraviolet procedure, but also facilitates precise titrimetry by allowing the addition of a major part of the EDTA solution from a weight buret with a visual indication of approach to the end point region.

Addition of ammonium purpurate to the solution to be titrated contributes an additional reaction to be considered in an accuracy evaluation. This situation may be examined from the following relationships, in which volume changes are neglected:

$$Ca^{++} + EDTA \rightleftharpoons CaEDTA$$

$$K_1 = \frac{[CaEDTA]}{[Ca^{++}][EDTA]}$$

$$Ca^{++} + Purpurate \rightleftharpoons CaPurpurate$$

$$K_2 = \frac{[CaPur]}{[Ca^{++}][Pur]}$$

Let T_A = total calcium ($[Ca^{++}]$ + [CaEDTA] + [CaPur])

T_E = total EDTA added ([EDTA] + [CaEDTA])

T_P = total purpurate ([Pur] + [CaPur])

Simultaneous solution of the above five expressions for the free calcium ion concentration gives Equation (1). Expressions for the other constitutents are fourth degree, and computation of their concentrations is more easily done by solution of Equation (1) and substitution of the result in Equations (2) and (3).

$$[Ca^{++}]^3 + \left(\frac{1}{K_1} + \frac{1}{K_2} + T_E + T_P - T_A\right)[Ca^{++}]^2 + \left(\frac{1}{K_1 K_2} + \frac{T_E}{K_2} + \frac{T_P}{K_1} - \frac{T_A}{K_1} - \frac{T_A}{K_2}\right) \cdot [Ca^{++}] - \frac{T_A}{K_1 K_2} = 0 \quad (1)$$

and

$$[Pur] = \frac{T_P}{1 + K_2[Ca^{++}]} \quad (2)$$

and

$$[CaPur] = T_P - [Pur] \quad (3)$$

The log formation constant for the reaction between calcium and EDTA is approximately 10.6 (Schwarzenbach and Ackermann 1948). The formation constant for the calcium purpurate complex has been examined (Schwarzenbach and Gysling 1949), and values of log K equals 4.1 at pH 10.1 and log K equals 4.8 at pH 11.5 were found. The results of calculations of free calcium ion and calcium purpurate concentrations from Equations (1), (2), and (3) for selected values of added EDTA are shown in Figure 1.

The accuracy which would result if the end point is estimated from photometric

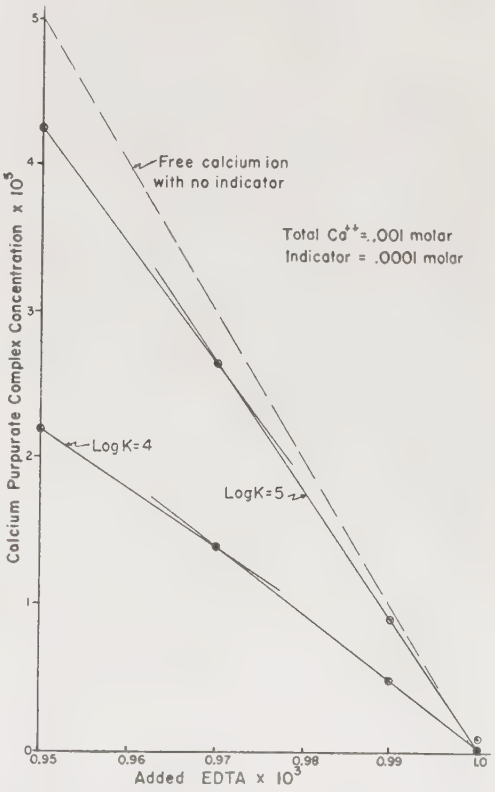

FIG. 1. Calcium purpurate complex versus added EDTA.

measurements of the calcium purpurate complex in the region (added EDTA equals 0.00097 molar to 0.00099 molar) may be estimated from the intercept of a straight line through the two points which bound this region. The intercept of this straight line is 0.00100049 molar added EDTA, so that calcium solutions whose concentration is one millimolar or greater may be titrated with a systematic error of 0.05 per cent or less. The shape of the curve indicates that use of measurements within the region will decrease the error, so that is is concluded that this photometric titration might be accurate to a few parts in ten thousand of the calcium present.

Experimental

The calcium purpurate complex has maximum absorption at 510 mμ at pH 12, and the purpurate ion also absorbs appreciably at this wavelength, so that a reference solution containing the same concentration of dye should be used. The dye decomposes with time and the reference solution provides partial compensation for the decrease in absorbance. With a light path length of 25 mm, the added EDTA region examined above will extend from 0.8 to 0.1 absorbance. Photometric measurements may be readily made with a precision of 0.5 per cent in this range. The use of a weight buret to add 97 per cent of the EDTA and a microburet for the final increments allows added EDTA to be determined with a preicision of 0.005 per cent or better, and hence the variance in the end point estimation is due to the photometric variance. The manner in which the photometric variance is reflected on the end point estimate is not readily evaluated, since the relation is a non-linear combination of stochastic variables. An upper and lower bound may be established from a choice of maximum positive and negative deviations of the measures from the mean values (Kinsman, personal communication). For the conditions considered above, deviations as large as 0.5 per cent will occur with a probability of not more than 0.03.

A titration vessel shown in Figure 2 was constructed by the writer and found to be convenient. The cell consists of a central

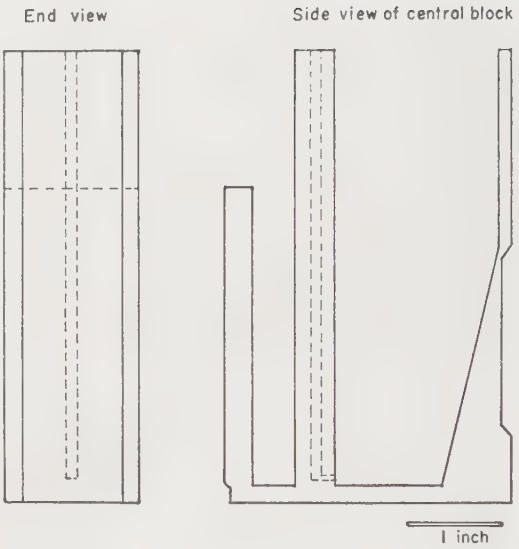

FIG. 2. Photometric titration cell.

block of lucite to which flat plates of lucite are attached by softening with ethylene dichloride. The solution to be titrated is rapidly mixed by a stream of gas bubbles, which enter at the bottom of the titration compartment. Volumes of 25 to 75 ml may be used in this cell, and the other ranges are readily available by variation of the thickness of the central block. No adsorption or chemical reactivity by the lucite was found for the solutions used in the calcium titrations.

Absorption measurements were made with a Beckman Model DU Spectrophotometer. The standard 1-cm cell compartment was modified by the addition of another 1-cm cell compartment with the slider removed (Beckman catalogue, number 2390). The portion of the cell that projects above this compartment was enclosed in a brass box whose front and back sides extend down to the lower edge of the compartment. The entrance of the microburet into the compartment was made light-proof by a small stand tube and rubber sleeve. The titration assembly is shown in Figure 3.

The reaction between calcium and EDTA ion has been shown to be stoichiometrical by several authors (Blaedel and Knight 1954, Hall *et al.* 1954, Sweetser and Bricker 1954) with accuracies within 0.1 to 0.05 per cent.

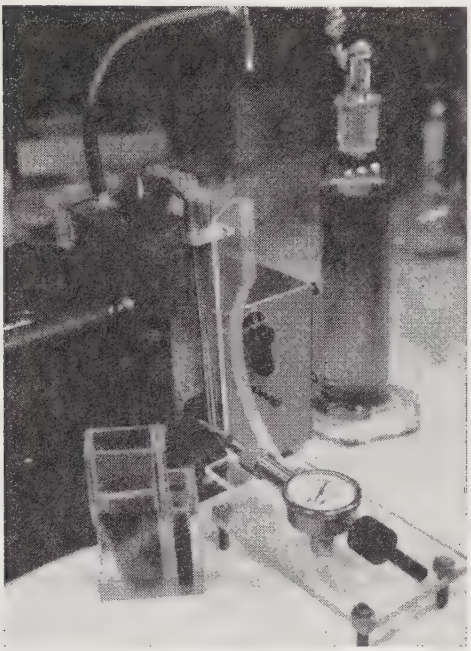

FIG. 3. Photometric titration assembly.

Conductance of the solutions and ultraviolet absorption of the excess EDTA were used to follow the course of the titration. The accuracy was restricted by the volumetric measurements rather than the instrumental determination of the constituents of the solutions. The stoichiometric relation was found to be one to one on a mole basis.

The use of the calcium purpurate complex in the manner described above is shown by the data presented in Table 1 to have no effect on the stoichiometric relation. If a straight line is fitted to the data of Table 1 by the method of least squares, the standard error of estimate is 0.0024g of EDTA solution. Solutions that require 24g of EDTA solution for titration may therefore be examined with a standard deviation of 0.01 per cent. The intercept of the best fit straight line is minus 0.0024g of EDTA solution, which is not a statistically significant difference from zero. If the purpurate procedure introduced a systematic error, it would be revealed in these data as a non-zero intercept. These data serve as a demonstration that the end point taken is the equivalence point.

TABLE 1. *Photometric titration of calcium solutions with EDTA*

Grams of calcium solution	Grams of EDTA solution
4.2925	8.692
5.6550	11.451
8.3056	16.816
8.3094	16.829
10.3845	21.029
14.2688	28.899
14.2702	28.901
14.2730	28.902

The calcium solution was prepared by dissolving Mallinckrodt Primary Standard Calcium Carbonate in redistilled hydrochloric acid and diluting with double distilled water. One gram of solution contained approximately 0.4 mg of calcium. The EDTA solution was prepared from reagent grade material and was approximately 0.005 molar. Redistilled piperidine was used to adjust the pH to 11.8.

Standardization of EDTA solutions

If the standardization of the EDTA can be done with precision and accuracy, the calcium content of the distilled water solutions may be determined with confidence. It is very difficult to obtain calcium salts that do not contain impurities of 0.01 per cent, in particular other members of Periodic Group IIa. Drying of the salt also presents a problem unless fusion in an inert atmosphere is used. The use of copper metal has the advantages that very pure electrolytic copper is readily available and is not hygroscopic. The purpurate complex with copper may be used for spectrophotometric titration at 450 mμ. Repeated titrations of copper standards with EDTA give a standard deviation of 0.02 per cent, which is comparable to the confidence in the values of the atomic weights of calcium and copper.

SEPARATION OF CALCIUM FROM OTHER PERIODIC GROUP IIA IONS

Ion exchange chromatography has been shown to be a powerful and operationally simple method for utilizing small differences in chemical affinity, as evidenced by the separation of the rare earths (Tompkins *et al.* 1947). Ion exchange has been used for the separation of calcium from magnesium in limestones and dolomites (Campbell and Kenner 1954). The separation of calcium,

strontium, and barium using ammonium lactate as an eluting agent has been described (Lerner and Rieman 1954). However these procedures do not yield an eluate suitable for precise titrimetry.

Ion exchange chromatographic separation of magnesium, calcium, and strontium has been investigated by the author. The ideal eluting reagent should form relatively strong complexes with these elements and show a large difference in complexing ability toward these elements in order that sharp separations may be obtained. Dicarboxylic acids, hydroxy acids, and amino acids form strong complexes with these elements but do not have large differences in affinity between these elements (Martell and Calvin 1952). Acetylacetone (pentanedione-2,4) forms a complex with magnesium which has a log-formation constant for the one to two complex of 5.9 (Van Uitert *et al.* 1953). The calcium complex is much weaker, and the log-formation constant is estimated by the author to be 1.2 by comparison with the affinity of calcium lactate solutions for an ion exchange resin. The strontium complex is weaker than the calcium, and the formation constant has not been determined.

The use of acetylacetonate ion as an eluting agent for magnesium is shown in Figure 4. Ammonium acetylacetonate is not very stable as shown by the change in elution with age of the solution. Under the conditions specified no calcium will appear in the effluent until 250 ml of this solution has passed through the column, so that a very clean separation is effected.

A sample of dolomite, Bureau of Standards Sample No. 88, was analyzed by solution in hydrochloric acid, dilution, uptake on a column, elution of magnesium with acetylacetone, elution of the calcium with 2 molar potassium nitrate, and visual titration with EDTA in the presence of Eriochrome Black T and a standard amount of magnesium. The reported value of the sample was 30.49 ± 0.02 per cent calcium oxide, and the ion exchange procedure gave 30.54 ± 0.05 per cent calcium oxide. The EDTA was standardized against a magnesium solution, whose magnesium content had been determined gravimetrically with oxine (Kolthoff and

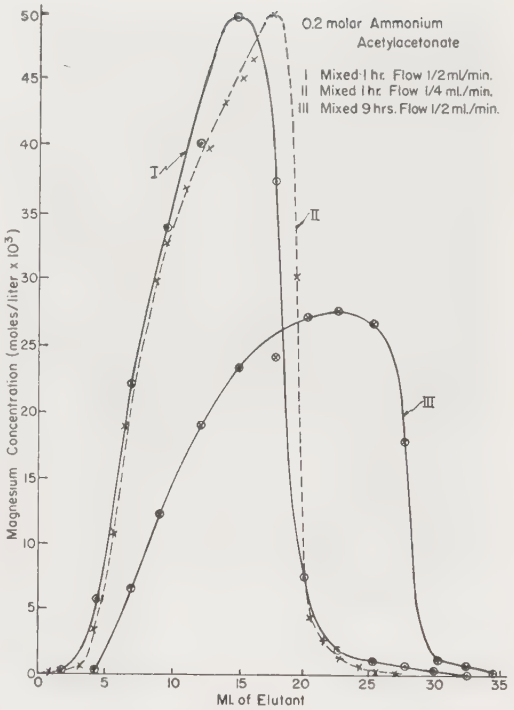
0.2 molar Ammonium Acetylacetonate
I Mixed 1 hr. Flow 1/2 mL/min.
II Mixed 1 hr. Flow 1/4 mL/min.
III Mixed 9 hrs. Flow 1/2 mL/min.
Magnesium Concentration (moles/liter x 10³)
ML of Elutant
I
II
III

FIG. 4. Elution of magnesium with ammonium acetylacetonate. Total magnesium ca. 12 mg. Column Dowex 50 (100–200 mesh, 8% cross-linkage) 8 by 80 mm.

Sandell 1943), and volumetrically after exchange for hydrogen ion and titration with sodium hydroxide, which had been standardized with potassium hydrogen phthalate. This sample of dolomite contained less than 0.01 per cent strontium, which was negligible.

Acetylacetone forms a weak complex with calcium and may be used as an eluting agent. Four milligrams of calcium can be removed from an 8 x 80 mm column of Dowex 50 (100–200 mesh, 8 per cent cross linkage) with 27 ml of 1.0 molal ammonium acetylacetonate. When one milligram of strontium is placed on the same column, no strontium will appear in the first 60 ml of the 1.0 molal ammonium acetylacetonate solution. One milligram of strontium can be quantitatively removed from this column with 50 ml of 2 molal ammonium chloride. When pure calcium solutions, prepared from Mallinckrodt Primary Standard calcium carbonate were titrated before and after uptake on the column and elution, recovery

was complete to 0.02 per cent of the calcium present.

Aqueous solutions of acetylacetone are not very stable. When 50 ml of 1.0 molal ammonium acetylacetonate are heated at 95°C for 90 minutes, quantitative decomposition occurs, as shown by no reaction with magnesium Eriochrome Black T complex, which it readily decolorizes. The product has not been identified except with regard to complexing ability.

Ion exchange procedures for natural waters have the advantage that the initial concentration of the ion may vary widely without influence on the subsequent steps in the procedure. When several aliquots of a calcium solution were diluted to 10 ml and 100 ml before uptake on a column, quantitative recovery was found in either case. Adjustment of the volume of sample taken may be used to furnish eluted solutions of approximately constant concentration, so that high precision may be retained even for quite dilute solutions.

Reagents

Ammonium hydroxide, 2 molal. Prepare from scrubbed ammonia gas and redistilled water.

Acetylacetone, redistilled to remove acetic acid.

Ammonium acetylacetonate, 0.2 molal. Prepare 15 minutes before use by mixing acetylacetone and 2 molal ammonium hydroxide.

Ammonium acetylacetonate, 1.0 molal. Prepare as above.

Piperidine, redistilled.

Ammonium purpurate, 0.002 molal. Prepare by dissolving in redistilled water and add sufficient absolute ethanol to make final solution 70 per cent alcohol.

EDTA, 0.004 molal.

Ammonium chloride, 2 molal. Prepare from redistilled hydrochloric acid or hydrogen chloride gas and ammonia gas.

Procedure

Prepare a bank of ten columns (8 x 80 mm) of Dowex 50 (100–200 mesh, 8 per cent cross linkage), which has been washed by decantation with 5 portions of 5 molar HCl to remove iron and converted to the ammonium form with 3 rinses of 2 molal ammonium hydroxide. Wash the columns with 100 ml of 2 molal ammonium chloride before use. Weigh a suitable sample in an automatic pipet and transfer to the ion exchange column. The sample size should be adjusted so that 12 mg or less of magnesium, 4 mg or less of calcium, and 1 mg or less of strontium will be present on the column. These quantities would amount to approximately a 10 g sample for open ocean water. Four duplicate samples and a duplicate blank should be run simultaneously.

Elute the magnesium with 50 ml of 0.2 molal ammonium acetylacetonate at a flow rate of 0.5 ml/min. at room temperature.

Elute the calcium with 50 ml of 1.0 molal ammonium acetylacetonate as described for magnesium. Collect the eluate in 150 ml beakers, cover with watch glasses, and heat with infrared lamps for 90 minutes without boiling. After cooling, add 1 ml of piperidine and 2 ml of 0.002 molal ammonium purpurate solution. Add 0.004 molal EDTA solution from a weight buret until a visual end point is reached, which should be approximately 97 per cent of the required EDTA. Transfer to the titration cell with three 5 ml distilled water washes. Continue the titration with a 1 ml microburet that can be read to 0.0001 ml. Plot the absorbance at 510 mμ against added EDTA and determine the end point by visual fitting of a straight line. Blanks of the order of 0.05 g of EDTA solution should be found, and duplicates agree to 0.002 g.

The columns are washed with 50 ml of 2 molal ammonium chloride to remove the strontium and are ready for use again. The 4 samples can be run in approximately 8 hours.

CALCIUM CONTENT IN SEA WATER

The calcium content of open ocean water is not known with great confidence. The values determined by oxalate procedures must be corrected for the interference of strontium, which amounts to approximately three per cent. The value given in standard texts (Sverdrup *et al.* 1942, Thompson and Robinson 1932, Harvey 1955) is 0.00070 for

the strontium-chlorinity ratio. However, recent analyses (Odum 1951, Smales 1951, Chow and Thompson 1955b, Bowen 1956) do not confirm this value. Odum (1951) examined 160 samples from the Atlantic Ocean and found 0.00042 for the strontium-chlorinity ratio, using flame photometry after separation of calcium and strontium from the sea water. Chow and Thompson (1955b) examined 28 samples from the Arctic, Atlantic, and Pacific Oceans and found 0.00042 $\pm$ 0.00002 for the strontium-chlorinity ratio, using flame photometry directly on the sample. Smales (1951) found 0.0005 for five samples in the Atlantic Ocean. The use of activation analysis was recently reported (Bowen 1956) and a strontium-chlorinity ratio of 0.0044 was found for a sample from the English Channel.

It appears that the previously accepted value for the strontium content is too high and therefore the corrected calcium content too low. Chow and Thompson (1955a) examined 16 samples from the Gulf of Alaska and found a calcium-chlorinity ratio of 0.02184 by flame photometry. The previously accepted value (Sverdrup *et al.* 1942, Thompson and Robinson 1932, Harvey 1955) was 0.02152 corrected to 0.02106 in view of the strontium difficulty. The strontium correction which has been used appears to be incorrect both from new analyses of the strontium content of sea water and from new analyses of the calcium in sea water.

The author had an opportunity to analyze some sea water samples collected by the U. S. Geological Survey in the Bahamas Bank area. Two of the samples were examined by four different methods and analysts. Table 2 is reproduced from data in a proposed U. S. Geological Survey professional paper on the Bahamas Bank region. Sample *It'* is open ocean water, and Sample *Al'* is from a region of active precipitation of calcium carbonate on the Bahamas Bank.

The strontium corrections for the triple oxalate procedure were found by flame photometric analysis of the precipitate from a parallel determination. The strontium content found indicates that strontium is not quantitatively retained by the particular procedure used and that gravimetric analyses for calcium can not be corrected by the strontium content of the original sample without due regard for the particular method used.

Table 2. *Comparative calcium analyses of sea water*

Analytical method	Sample	Cl ‰	Calcium (mg/kg)		Magnesium (mg/kg)	
			observed	calculated from Cl	observed	calculated from Cl
Ion exchange	*It'*	19.94	423.1	419.9	1330	1335
Photometric titration	*Al'*	23.12	476.9	486.9	1542	1548
Triple oxalate	*It'*		424 (corrected for 4 mg strontium)			
	Al'		476 (corrected for 5 mg strontium)			

These same samples were examined by direct visual and photometric titrations with EDTA. These procedures gave results as much as four per cent too low compared to the results of the oxalate procedure, and variances of one per cent.

The data of Table 2 show the agreement between the classical oxalate procedure and the method proposed by the author. The differences found are expected from the precision of the oxalate method.

Sample *It'* from the open ocean has a higher calcium content than is calculated from the chlorinity and the calcium-chlorinity ratio previously accepted. If the newer determinations of strontium are correct as implied above, the previously accepted analyses for calcium should be corrected to a calcium-chlorinity ratio of 0.02126 from the 0.02152 value, rather than to the 0.02106 value previously accepted. The calcium-chlorinity ratio of sample *It'* is 0.02122, in fair quantitative agreement with the idea that the older strontium determinations were in error.

Samples from the Chesapeake Bay and its tributaries have been analysed by the proposed method (Carpenter 1957). The calcium content of these samples may be predicted best from the chlorinity and the

river-water and sea-water calcium contents if a calcium-chlorinity ratio of 0.02119 for the sea-water source for this region is assumed, which again indicates that the previously accepted value for the calcium content of sea water is too low.

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The Estimation of Phytoplankton Production in the Ocean from Chlorophyll and Light Data[1]

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ABSTRACT

The relationship was determined between photosynthetic rate at light saturation and chlorophyll content in natural populations and cultures of marine phytoplankton. A mean value of 3.7 g C assimilated/hour/g chlorophyll was obtained from the experimental results and from data present in the literature.

A method is described by means of which organic production beneath a unit of sea surface may be estimated from the chlorophyll content of the water, the total daily solar radiation reaching the sea surface, and the extinction coefficient of visible light in the water column. Production values calculated by this method are compared with values obtained by *in situ* productivity measurements.

INTRODUCTION

In an earlier paper (Ryther 1956a) a relationship was described between the photosynthesis of phytoplankton populations and the amount of solar radiation falling on the surface of the ocean throughout a day. A graph was constructed, reproduced here as the broken line in Figure 1, showing relative photosynthesis beneath a square meter of sea surface for different values of total daily radiation incident to the sea surface. The value for photosynthesis (the right-hand ordinate in Figure 1), when divided by the extinction coefficient of visible light in the water column in meters (k), gives photosynthesis beneath a square meter of sea surface per day relative to the rate of photosynthesis per cubic meter per hour at light saturation.

For situations in which phytoplankton populations are distributed homogeneously with depth, an absolute value of daily photosynthesis by the entire phytoplankton population may be obtained from the expression

$$P = \frac{R}{k} \times p(\text{sat.}) \qquad (1)$$

where

P = photosynthesis of the phytoplankton population in g carbon/m^2/day,
R = relative photosynthesis from Figure 1 (broken line) for the appropriate value of surface radiation,
k = extinction coefficient, per meter, as measured,
p (sat.) = photosynthesis of a sample of the population at 2000 foot-candles, as measured, in g carbon/m^3/hour.

If the phytoplankton are not distributed evenly with depth, photosynthesis must be calculated separately for several depths considering both the standing crop and the light at each depth. The solid lines in Figure 1 show daily photosynthesis per cubic meter (the left-hand ordinate) relative to photosynthesis per cubic meter per hour at light saturation at the sea surface (I_0) and at the depths where the surface light intensity is reduced to 50%, 25%, 10%, and 1% respectively. An absolute value for daily photosynthesis at each of these depths may be obtained from the expression:

$$P_d = R_d \times p_d \text{ (sat.)} \qquad (2)$$

where

P_d = daily photosynthesis (g carbon/m^3) at depth (d),

[1] Contribution No. 902 from the Woods Hole Oceanographic Institution. This work was carried out with the assistance of research grants from the National Science Foundation.

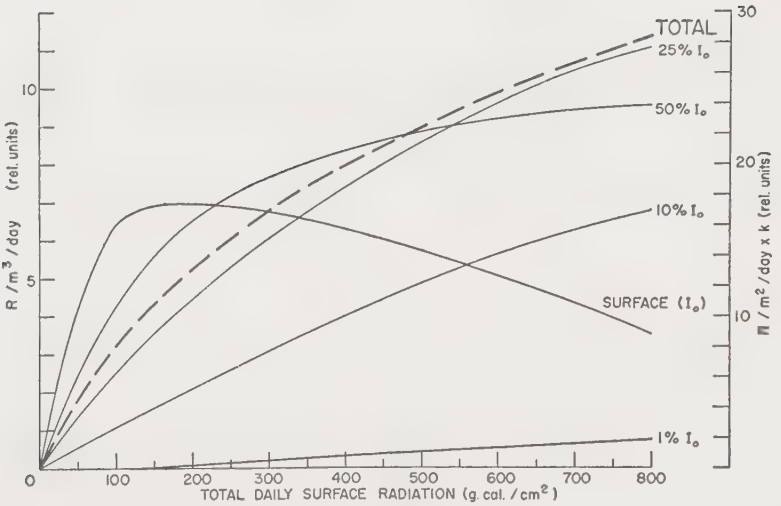

FIG. 1. The relation between total daily surface radiation and daily relative photosynthesis (R) beneath a unit of sea surface (broken line) and at the depths where the surface light intensity is reduced by specified amounts (solid lines).

R_d = relative photosynthesis at depth (d) from Figure 1 for the appropriate value for surface radiation,

p_d (sat.) = photosynthesis of a water sample from depth (d) at 2000 foot-candles, as measured, in g carbon/m³/hour.

Daily photosynthesis beneath a square meter of sea surface may then be calculated by graphic integration of the values so obtained for each depth.

The method described above, in either of its variations, represents a decided improvement over the alternative of collecting water samples from a series of depths and resuspending them at these depths for whatever period of time is necessary to detect measurable photosynthesis. However, even the practice of carrying out measurements on shipboard under constant illumination leaves much to be desired. Temperature control becomes a problem, particularly if samples from several depths of a thermally stratified water column are being measured at once. But the principle difficulty arises from the fact that no satisfactory experimental technique exists for measuring photosynthesis in the oligotrophic waters of the open ocean, even under controlled illumination. The uptake of C^{14} (Steemann Nielsen 1952), cannot be interpreted in terms of total photosynthetic rates without accompanying measurements of respiration (see Ryther 1956b). The measurement of oxygen production by means of the "light and dark bottle" technique cannot be detected in experiments of less than several days, and the interpretation of values so obtained is equally questionable. Consequently the authors have turned to chlorophyll *a* as an index of photosynthesis, and have attempted to relate photosynthesis at light saturation to the chlorophyll content of the phytoplankton.

THE RELATIONSHIP BETWEEN CHLOROPHYLL AND PHOTOSYNTHESIS AT LIGHT SATURATION

A rather constant relationship between chlorophyll and photosynthesis at any given light intensity has been described by several authors who have worked both in the field (Manning and Juday 1941, Gessner 1949, Edmondson 1955) and in the laboratory (Fleischer 1935, Emerson *et al.* 1940, Blaauw-Jansen *et al.* 1950, Ryther 1956b). In these studies, the relationship appears to hold constant for different species (Edmondson, Ryther) and within a wide range of chlorophyll content of the organisms resulting from their state of nutrition (Fleischer) or the duration or intensity of light to which

they had been previously exposed (Blaauw-Jansen *et al.*, Ryther).

A series of measurements were made by the present authors of chlorophyll *a* content and rate of photosynthesis at light saturation of natural phytoplankton populations occurring in the coastal waters around Woods Hole, Massachusetts.

Chlorophyll *a* was determined by filtering 1–3 liters of sea water through a millipore filter and extracting the pigments with acetone (Creitz & Richards 1955). Light absorption of the acetone extract was measured at selected wavelengths in a Beckman Model DU Spectrophotometer according to the methods of Richards with Thompson (1952), and chlorophyll *a* calculated from the nomographs of Duxbury and Yentsch (1956).

Photosynthesis measurements were made under 2000 foot-candles of fluorescent illumination in an incubator cooled by running sea water which kept the experimental vessels within 1°C of the water from which the samples had been taken. Photosynthesis was determined from the difference in dissolved oxygen concentration (Winkler method) in triplicate series of transparent and darkened 30-ml glass-stoppered bottles after 24 hours. The photosynthesis: chlorophyll ratio is expressed as grams carbon assimilated per hour per gram chlorophyll *a*. Carbon assimilation was calculated from evolved oxygen assuming a photosynthetic quotient $\frac{(+O_2)}{(-CO_2)}$ of 1.25 (see Ryther 1956b).

Measurements were made during the period March–July, 1956, at times when the water temperature was 10°, 15° and 20°C, respectively. The mean ratio (photosynthesis:chlorophyll) and standard deviation of approximately 10 measurements at each temperature is given in Table 1. Also included in this table is the mean ratio of 45 measurements made throughout the year between March, 1956, and February, 1957. Although winter values tended to be lower, indicating a temperature effect, the trend was not clear enough to permit a temperature correction. A series of anomalous results, giving ratios 10–20 times those reported in Table 1 was obtained with

TABLE 1. *The ratio of photosynthesis at light saturation to chlorophyll (g carbon assimilated/hour/g chlorophyll) in phytoplankton populations and cultures*

Source	Reference	No. measurements	Temp. (°C.)	*R*	
				Mean	Std. dev.
Coastal water (Woods Hole)	Ryther & Yentsch				
March–April		15	10	3.4	2.8
May		7	15	5.2	0.9
June–July		9	20	5.2	2.5
Annual average		45		5.7	2.8
Cultures—marine phytoplankton	Ryther & Yentsch	18	10	2.8	2.1
		7	15	4.8	0.8
Georges Bank	Riley (1941)	Annual mean		3.0	
Wesslingsee, Bavaria	Gessner (1949)	Annual mean		3.0	
Wisconsin lakes	Manning & Juday (1941)	19		2.1	1.8
Mean				3.7	

natural populations from two salt ponds, Eel Pond in Woods Hole and Great Pond in Falmouth, Massachusetts. The phytoplankton in these ponds was characterized by an abundance of small, green algae. It is believed that the high values resulted at least in part from the inability to extract completely the pigments from these cells. These atypical results have not been included here.

Similar measurements were made with cultures of marine phytoplankton including the diatoms *Skeletonema costatum*, *Amphora perpusilla*, *Navicula* sp., *Nitzschia palea*, and *Nitzschia closterium* (*Phaeodactylum tricornutum*); the green flagellates *Dunaliella euchlora* and *Chlamydomonas* sp.; the dinoflagellate *Prorocentrum micans*; and the chrysophyte *Olisthodiscus* sp.

The cultures were routinely grown at 15°C under approximately 1000 foot-candles of fluorescent illumination. Prior to the experiments the cultures were diluted with enriched sea water so that the cell suspensions did not appreciably reduce the light intensity. Mean values and standard deviations for all species at 10° and 15° are given in Table 1. The low ratio at 10° may have resulted in part from the fact that the organisms were not previously conditioned

TABLE 2. *A comparison of some* in situ *productivity measurements with values estimated from chlorophyll, radiation, and extinction coefficient*

Place	Reference	Surface radiation (g cal./cm²/day)	k	Chlorophyll (mg/m³)	Calculated production (g C/m²/day)	Measured production (g C/m²/day)
N. W. Atlantic	Riley (1939)					
4 northern stations		460[1]	0.10	2.1	1.67	0.70
14 tropical stations		460[1]	0.05	1.0	1.57	1.18
Long Island Sound	Riley (1956) Conover (1956)					
March–May, '52		400	0.46	7.2	1.17	1.06
Feb.–March, '53		252	0.73	11.8	0.90	1.32
Aug.–Nov., '53		347	0.63	5.6	0.61	0.95
Friday Harbor, Washington	Yentsch (unpubl.)	300	0.13	1.0	0.48	0.34
East Sound, Washington	Yentsch (unpubl.)	700	0.30	15.0	4.80	5.10
Gulf of Alaska	Yentsch (unpubl.)	229[1]	0.10	2.5	1.34	1.50
Woods Hole Harbor	Ryther & Yentsch	550	0.27	3.35	1.05	1.63
		480	0.27	1.69	0.51	0.79
		610	0.24	2.80	1.04	1.10

[1] Estimated from Kimball (1928).

to that temperature before the start of the experiments.

Also included in Table 1 are photosynthesis:chlorophyll ratios from a series of Wisconsin lakes (Manning and Juday 1941), the Wesslingsee (Gessner 1949) and George's Bank (Riley 1941). The ratios compiled in the table are in such close agreement that the mean from all sources has been taken as a working value, which is 3.7 g carbon/hour/g chlorophyll.[2]

THE ESTIMATION OF PRODUCTION FROM RADIATION, TRANSPARENCY, AND CHLOROPHYLL

Assuming now that 3.7 g carbon are assimilated per hour at light saturation for each gram of phytoplankton chlorophyll, Equation (1) may be written

$$P = \frac{R}{k} \times C \times 3.7 \qquad (3)$$

where

C = g chlorophyll/m³ in a sample of a homogeneously distributed population.

Similarly Equation (2) may be written

$$P_d = R_d \times C_d \times 3.7 \qquad (4)$$

[2] This is equivalent to an assimilation number (g/CO_2 assimilated/hour/g chlorophyll) of 13.6.

where

C_d = g chlorophyll/m³ at depth (d).

In Table 2 are given some production values obtained from *in situ* "light and dark bottle" oxygen experiments (Yentsch, Ryther and Yentsch), and from *in situ* changes in dissolved oxygen in the water column or a combination of the two methods (Riley). For each value are also given data for chlorophyll, daily surface radiation, and extinction coefficient, measured or estimated as indicated in the table. Except in the case of Woods Hole Harbor, where the phytoplankton are known to have been evenly distributed with depth, the vertical distribution of chlorophyll is not known. Consequently production has been calculated from Equation (3), assuming even vertical distribution, though this was probably not true in every case. The calculated values are given in Table 2 beside those obtained from *in situ* measurements.

DISCUSSION

There is, on the whole, close agreement between production values calculated by the method outlined above and those obtained from *in situ* rate measurements. This agreement, which is consistent for situations in which production varies by an

order of magnitude, represents the best approximation of a critical test of the method. However, it should be pointed out that production values calculated from long term *in situ* bottle experiments are, themselves, subject to severe criticism, and, as a consequence, disagreement between values obtained by the new method and the earlier estimates does not necessarily represent the inadequacy of the former.

Probably the weakest point in the foregoing treatment is the photosynthesis-chlorophyll factor. There is little reason to assume that this must be constant under all conditions. The presence of inactive chlorophyll in plants, as reported by Smith (1954) and others, and the findings by Gillbricht (1951) and Krey (1952) of relatively large quantities of chlorophyll associated with non-living detrital material indicate that the pigment is not an infallible indicator of photosynthetic capacity. However, much of the error from these sources may be circumvented by using precise, narrow band spectrophotometric analyses, as referred to above, since neither the precursors nor the degradation products of chlorophyll have exactly the same absorption characteristics as the active pigment. The fact that the chlorophyll concentration of phytoplankton varies diurnally in nature (Yentsch and Ryther 1957) further complicates the picture. There is undoubtedly some temperature effect. Finally, the relationship between chlorophyll and photosynthesis undoubtedly shows some variability between the major groups and perhaps even between species of plankton algae. The need is apparent for a more detailed study of this relationship which would perhaps allow the investigator to take into account such factors as temperature, light, season, time of day, or species composition in refining the value suggested above. However, much of the variability in the photosynthesis: chlorophyll ratio indicated in Table 1 is undoubtedly due to errors in the measurement of both of these variables by standard field methods, particularly at their lower limits of detectability—errors which are compounded by taking their quotient.

Despite these errors and any true variability in the photosynthesis:chlorophyll ratio, it seems unlikely in view of the existing evidence that a correct ratio under most conditions differs from that suggested above by more than a factor of two. When one considers that both radiation and chlorophyll concentration fluctuate in nature by more than an order of magnitude it is obvious that any departure from the mean photosynthesis:chlorophyll ratio employed above will be of relatively minor significance in affecting the calculated value for production.

The obvious advantage of this method is that it completely eliminates the necessity for making time studies of photosynthesis or other related changes which are awkward, time consuming, and, in the case of oligotrophic ocean waters, unreliable. For routine survey work, average radiation data for any date and latitude may be obtained from the tables of Kimball (1928). If a submarine photometer is not available, the extinction coefficient may be estimated from Secchi disc readings (see Poole and Atkins 1929). Filtered chlorophyll samples may be stored for a period of months if properly treated (Creitz and Richards 1955) and the determinations carried out ashore. As a consequence, productivity measurements may now be made a routine phase of oceanographic investigation requiring a minimum of time, expense, and interference with other activities.

Algal cultures used in the experiments described above were originally obtained from Dr. B. M. Sweeney (*Prorocentrum micans*), Dr. L. Provasoli (*Amphora perpusilla*, *Nitzschia palea*, *Olisthodiscus* sp.), and Dr. R. Lewin (*Skeletonema costatum*). The other species were isolated in this laboratory. The authors gratefully acknowledge the technical assistance of Mrs. R. W. Kahler.

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Potassium Concentrations in Fertilized Trout Lakes[1]

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ABSTRACT

A series of experiments was performed on a group of six Michigan trout lakes to determine if biological productivity could be increased by the application of commercial fertilizers to the lakes. Measurements of potassium concentrations in the lakes before and after fertilization indicated that the potassium went into solution in proportion to the amounts of fertilizer added and gradually subsided from epilimnial waters while increasing in hypolimnial waters. Residual effects of fertilization on increased potassium concentrations in both epilimnial and hypolimnial waters were evident for nearly one year following fertilization. Bottom muds showed no marked increases in adsorbed potassium following fertilization. Adsorptive capacities of muds consisting primarily of inorganic materials were considerably greater than organic muds. Intensive phytoplankton blooms occurred following fertilization, but the potassium in the fertilizer probably did not contribute to the blooms because the amounts of potassium put into the lakes were only a small fraction of naturally occurring amounts already present in the lake waters.

INTRODUCTION

To determine the practicability of improving fishing in trout lakes by the application of commercial fertilizers, a series of experiments was initiated in 1946 by Professor Robert C. Ball, of Michigan State University, and continued under his direction by Tanner (1952) and Barrett (1953). Early results of these experiments, although promising (Ball 1950), furnished evidence that rapid or even undesirable eutrophication may occur if the rate of fertilizer application is not carefully controlled. In one phase of these experiments Tanner (*loc. cit.*) obtained significant increases in production of trout and fish food organisms following fertilization of four of six experimental trout lakes. In these same experiments significant decreases in lake-water alkalinities and marked vertical shifts in thermal strata followed fertilization. Barrett (*loc. cit.*), who measured phosphorus and potassium contents of the waters of these lakes during the experiments, found that the rate of disappearance of the added phosphorus from epilimnial waters was greater where alkalinities were above 120 ppm M.O. alkalinity. In hypolimnial waters where organic matter contents were high, this trend was reversed; phosphorus concentrations were correspondingly greater with increasing alkalinities.

General morphometric and biotic features of the six lakes included in these studies have previously been reported by Eschmeyer (1938) and the above authors. The lakes are all nearly circular in outline, they range in maximum depth from 35 to 72 feet, and none is larger than 6 acres in area. In general, the deeper the lake the greater the alkalinity. The lakes are believed to be limestone-sink basins, and are located within the Pigeon River Trout Research area in Otsego and Cheboygan Counties, Michigan. The lakes included in the studies and their respective methyl-orange alkalinities (determined in 1948) are: North Twin 34 ppm, South Twin 74 ppm, West Lost 138 ppm, Hemlock 163 ppm, Lost 177 ppm, and Section Four 192 ppm.

PROCEDURE AND METHODS

Commercial fertilizer consisting of nitrogen, phosphorus, and potassium in the ratio of 6:10:4 was applied to West Lost, Hemlock, Lost, and Section Four lakes during the months of June and July in 1949 and 1950. The two softer-water lakes, North

[1] Acknowledgment is due Dr. R. C. Ball, Dr. H. A. Tanner, Dr. A. Tucker, and the Michigan State University All-University Research Committee for invaluable aid and assistance. Contribution Number 66 from the Department of Natural Science.

TABLE 1. *Concentrations in ppm of potassium added to the lakes*[1]

Period of application	West Lost	Hemlock	Lost	Section Four
1949 June 16–18	0.0368	0.056	0.115	0.146
July 7–8	0.0184	0.031	0.054	0.073
July 27–28	0.0184	0.031	0.054	0.073
1950 June 14–16	0.0184	0.031	0.054	0.073
July 7–8	0.0184	0.031	0.054	0.073
July 27–28	0.0184	0.031	0.054	0.073
Total	0.1288	0.211	0.385	0.511

[1] ppm is the ratio of amount of potassium fertilizer to the computed volume of water in the lakes.

and South Twin, were left unfertilized as controls. Applications of fertilizer were made at three-week intervals beginning in mid-June and continuing until the end of July. Amounts of potassium applied to the lakes are listed in Table 1. Water samples for chemical analysis were collected with a Kemmerer water bottle at the centers of the lakes from the surface and from the lower hypolimnion within three feet of the bottom. Mud samples were collected with an Ekman dredge from the lake bottoms. As recommended by Zobell and Brown (1944), water samples were preserved with chloroform to inhibit bacterial activity until such time that analysis for phosphorus and potassium could be done. The mud samples were air-dried in a water bath at 60°C and then pulverized and screened by a number 35 grade sieve. Adsorbed potassium was extracted from the dried mud samples by adding 7 ml of a 25% sodium nitrate solution to 1.0 g of mud in a vial. The vial was shaken vigorously by hand for 60 seconds, and its contents filtered. This filtrate, as well as the lake-water samples, were analyzed for soluble potassium according to the technique of West, Folse, and Montgomery (1950). A Beckman flame photometer and DU Spectrophotometer were used for these analyses. Organic matter contents of the muds were determined by the wet combustion procedure as described by Peech *et al.* (1947).

RESULTS AND DISCUSSION

The information in the literature about the requirements, utilization, and availability of potassium as a nutrient in freshwater lakes is not too plentiful. Lohammar (1938) in a study of 167 lakes in Sweden found that potassium contents ranged from less than 0.2 ppm to over 20.0 ppm. Most lakes, however, contained from 0.2 ppm to 3 ppm of potassium. Lohuis, Meloche, and Juday (1938) reported a range from 0.24 to 2.39 ppm in 91 Wisconsin lakes. Höll (1951) found for a number of central European lakes that potassium values below 1.0 ppm were generally typical of oligotrophic lakes, and that as values increased above 1.0 ppm increasingly higher trophic conditions existed. Waters of three southern Michigan lakes examined by the present author contained the following potassium concentrations: Burke 1.0 ppm; Park 2.8 ppm, and Wintergreen 4.4 ppm. The six Pigeon River lakes of the present study contained from 0.5 to 0.8 ppm prior to fertilization. In these nine Michigan lakes the relation of the potassium content to the trophic level is the same as Höll found, there being greater potassium contents in lakes with greater eutrophy.

Neess (1949) in a review of fertilization studies in Europe reported that some investigators believed potassium fertilizers to be most important in ponds located in sandy, potassium-poor areas. Most studies in Neess' review, however, seem to have been too inconclusive for definite decisions regarding the role of potassium as a limiting factor in ponds. Rodhe (1948) investigated optimum nutritional levels of potassium for the alga *Scenedesmus quadricauda* in laboratory cultures and concluded that this nutrient is not an important limiting factor of this alga in fresh water. Höll (*loc. cit.*) came to the conclusion that normally existing potassium contents are sufficient for the growth of phytoplankton in most lakes. The present author arrived at a similar conclusion after measuring the effect of potassium on the growth of algae in some laboratory experiments. A large sample of water was collected from Burke Lake, Michigan, and subdivided into 12 one-liter size samples, each of which was treated with nitrogen (as a nitrate) and phosphorus so that the nitrogen and phosphorus contents of each of the samples were increased by

8 ppm and 4 ppm, respectively. Different amounts of potassium were added to these samples so that the potassium concentrations in the samples were increased by amounts ranging from 0.0 ppm to 32 ppm. After exposure to sunlight for 21 days in a greenhouse all samples showed a marked growth of algae; there was, however, no correlation between amounts of potassium in the cultures and the growth of algae.

Epilimnial waters

In the present study on the chemical nature of the waters of the Pigeon River Lakes, some information has been provided on the effects of increasing available potassium in natural lakes by fertilization. In this series of experiments the amount of potassium added to a lake at any one time was very small compared to the amount naturally existing in the lake at that time (Tables 1 and 2). For example, the greatest single application made was 0.146 ppm (to Section Four Lake in June 1949). The value 0.146 is the ratio of the potassium added to the computed volume of water in the lake. Just prior to the application of this fertilizer, Section Four Lake contained 0.7 ppm of potassium. At the end of the summer, however, after three successive applications of fertilizer, the potassium contents of the four fertilized lakes showed increases over prefertilization levels by amounts ranging from 0.2 to 0.8 ppm. The amount of increases in the lakes were proportionately related to amounts added. During the same period the control lakes decreased in potassium contents. These data support the conclusion that the inorganic potassium fertilizer went into solution and remained available to algae as a nutrient for a period of at least several weeks. By contrast, it is known that phosphorus is quickly removed from solution when added in the inorganic form to lakes.

TABLE 2. *Concentrations in ppm of soluble potassium in epilimnial water samples*

Period of analysis	Control lakes		Fertilized lakes			
	North Twin	South Twin	West Lost	Hemlock	Lost	Section Four
1949 May 1	0.6	0.8	0.6	0.7	0.5	0.5
June 15	0.6	—	0.4	0.6	0.7	0.7
June 18–23	0.4	—	0.6	0.6	0.6	0.9
July 27–28	0.4	—	0.9	0.8	1.0	1.2
July 29–30	0.7	—	0.8	—	1.3	1.4
Aug. 22–23	0.4	0.7	1.0	0.9	1.0	1.3
Average	0.52	0.75	0.72	0.72	0.85	1.00
1950 June 15	0.6	1.2	1.2	1.1	0.9	1.1
June 18–19	1.5	0.6	2.0	1.6	2.0	—
July 6–7	2.0	2.0	0.4	1.3	0.9	2.1
July 10–11	1.2	1.6	1.0	1.8	1.9	1.8
July 29–30	—	—	2.2	2.2	0.7	2.1
July 31–Aug. 1	0.1	1.1	1.3	1.1	1.2	0.9
Aug. 28–29	0.6	0.7	0.7	0.9	0.9	1.2
Average	1.00	1.20	1.26	1.43	1.21	1.53
1951 June 12–14	0.4	0.4	0.5	1.2	0.7	0.8
July 6–8	0.2	0.5	0.6	0.6	0.8	1.0
July 29–31	0.3	0.2	0.5	0.5	0.6	0.9
Aug. 19–21	0.4	0.2	0.5	0.5	0.6	0.6
Average	0.33	0.33	0.53	0.70	0.68	0.83

During the second summer of fertilization (1950) epilimnial potassium levels increased in both fertilized *and* unfertilized lakes to levels as high as 2 ppm. Because potassium and phosphorus (Barrett *loc. cit.*) increased in the hypolimnial (Table 3) as well as in the epilimnial waters in *both* fertilized and unfertilized lakes, causes other than the applied fertilizer must be found. It so happened that the spring snow thaws of 1950 were accompanied by unusually abundant and prolonged rainfalls. It is quite possible that this excessive rainfall carried large quantities of dissolved minerals into the lakes. The presence of increased quantities of these and other nutrients might explain the unexpected appearance of plankton blooms (Tanner *loc. cit.*) in these control lakes during the summer of 1950.

At the end of the second year of this study (August 1950), epilimnial potassium contents were very nearly equal to prefertilization levels plus total quantities added (Table 2). The potassium contents of the unfertilized lakes, however, were approximately the same as they had been in June 1949, two years previous. This gives further support to the conclusion that the fertilization program was responsible for increasing the quantities of available potassium in the experimental lakes.

In the summer of 1951 no more fertilizer

was applied, but water samples were collected from both fertilized and unfertilized lakes. At this time it was found that the lakes which had received the most fertilizer still contained, on the average, the highest potassium concentrations. It should be noted that the highest potassium contents occurred in those lakes having the highest alkalinities. Theoretically, one might expect *any* lake containing high carbonate alkalinities to also contain high concentrations of potassium. This relationship does not seem to occur, however, for prior to fertilization those lakes having highest alkalinities did not contain highest potassium concentrations. Höll (*loc. cit.*) in his studies on European lakes also found no close correlations between carbonate alkalinities and potassium contents of lake waters. This points to the conclusion that the higher potassium contents of the Pigeon River lakes after fertilization were indeed a result of the application of fertilizer. By the end of August 1951 epilimnial potassium concentrations had decreased to prefertilization levels.

These experiments give no evidence that potassium plays a critical role in limiting growth of primary food organisms in natural lakes. The planktonic algal responses to the fertilizer (as measured by Secchi disk visibility readings) were very great (Tanner *loc. cit.*). This response however probably cannot be attributed to the potassium in the fertilizer, because the fertilizer contained a negligible amount of potassium compared to the amounts already present in the lakes before fertilization. Although potassium contents increased in the fertilized lakes following fertilization, in no instance in 1949 were total potassium measurements of lake waters greater than 1.4 ppm. Averages for the four fertilized lakes in 1949 ranged from 0.72 to 1.00 ppm (Table 2). Thus the potassium of these lakes at this time was on the same order of magnitude as the average for most natural lakes as indicated by the data of the several studies already cited. For example, the data of Lohuis, Meloche, and Juday (*loc. cit.*) show that of 91 Wisconsin lakes, 43 contained 0.9 ppm or more of potassium, and only 13 of the 91 contained less than 0.5 ppm. In the lakes of the Pigeon River area algal growth responses to the fertilizer were very great. Since this growth occurred at potassium concentrations which averaged less than 1.00 ppm, support is given the conclusion that most lakes contain sufficient potassium for luxuriant algal growth, assuming other factors such as nitrogen and potassium are optimum.

Hypolimnial waters

Concentrations of potassium in the lower hypolimnial waters were approximately the same in both control and experimental lakes during the first year of fertilization (Table 3), with the control group ranging from an average of 0.80 ppm (North Twin) to 1.00 ppm (South Twin), and the fertilized group ranging from an average of 0.70 ppm (Section Four) to 1.25 ppm (Hemlock). Hypolimnial waters thus were only slightly higher in potassium concentrations than

TABLE 3. *Concentrations in ppm of soluble potassium in hypolimnial water samples*

Period of analysis		Control lakes		Fertilized lakes			
		North Twin	South Twin	West Lost	Hemlock	Lost	Section Four
1949	June 15	0.9	—	0.7	—	—	0.8
	June 18–22	0.6	—	1.1	2.2	0.8	0.7
	July 27–28	0.8	—	0.8	0.8	0.8	0.5
	July 29–30	1.0	—	1.1	1.1	1.0	0.7
	Aug. 22–25	0.7	—	1.0	1.0	1.0	0.7
	Aug. 29	0.8	1.0	0.7	—	—	0.8
	Average	0.80	1.00	0.90	1.25	0.90	0.70
1950	June 13–16	—	1.2	1.0	2.3	0.9	1.0
	June 18–19	1.6	1.4	2.0	—	1.6	1.4
	July 6–7	1.8	3.3	1.9	3.0	2.0	2.3
	July 10–11	1.5	1.3	1.8	3.1	1.8	1.6
	July 30	—	—	1.9	3.2	1.9	2.0
	July 31–Aug. 1	1.7	1.3	0.9	2.3	0.9	0.8
	Aug. 28–29	0.5	0.9	0.8	1.5	1.0	1.2
	Average	1.42	1.53	1.47	2.57	1.46	1.47
1951	June 12–14	0.4	0.6	0.8	2.0	1.5	1.1
	July 6–8	0.7	0.7	1.0	1.7	0.8	1.3
	July 27–31	0.8	1.3	1.1	0.7	—	1.5
	Aug. 19–21	0.6	0.9	1.8	2.2	2.5	1.0
	Average	0.63	0.88	1.18	1.65	1.60	1.23

were epilimnial waters at that time. During the second year of fertilization all lakes including controls made considerable increases in hypolimnial potassium, the control lakes averaging about the same as the experimental lakes. These data may be explained by the unusual abundance of rainfall of that year. Hypolimnial concentrations of the following year (1951), *i.e.* the year following cessation of fertilization, were higher in fertilized lakes than in control lakes, although increases were only approximately in proportion to amounts of fertilizer added. For example, the average values in 1951 of the control lakes were 0.63 ppm and 0.88 ppm, whereas the averages of the fertilized lakes ranged from 1.18 ppm to 1.65 ppm (Table 3). If these higher hypolimnial contents in fertilized lakes were in fact caused by the fertilizer applications, as seems likely, then it remains to be shown whether such increases were due to latent and gradual dissolution of the added potassium compounds, or perhaps to gradual subsidence from upper to lower waters. On the other hand, the potassium increases in the lake waters might have been due to secondary effects, such as increased biological decomposition of the organic substrate brought on by nitrogen and phosphorus additions. The latter postulate may also explain the potassium increases occurring in the epilimnion.

Bottom muds

Mud samples collected from the lake bottoms near the center of each lake were analyzed for adsorbed potassium contents (Table 4). Samples were taken in June 1949 just prior to the first application of fertilizer and in August 1949, 1950, and 1951. Determinations were made both of quantities of adsorbed potassium per unit weight of dried mud and of amounts of adsorbed potassium per unit volume of wet mud. It was necessary to make the laboratory analysis of adsorbed potassium on the muds after having dried them, because in the wet state different samples of muds contained large variations in water content. The use of dried muds in these analyses made it possible to measure more precisely the amounts of adsorbed potassium in samples which were gravimetrically comparable. After the amounts of adsorbed potassium were determined in units per unit weight of dried mud, they were converted to amounts of adsorbed potassium per unit volume of wet mud according to the following procedure. A 100 cc sample of wet mud was dried to constant weight in a water bath at 60°C. The resulting weight of the dried mud was recorded as the number of grams per cc of wet mud. The amount of adsorbed potassium was then determined and recorded as the number of micrograms per gram of dried mud. The micrograms of adsorbed potassium per cc of wet mud was then computed by multiplying the amount of potassium in the dried mud by the weight of dried mud per cc of wet mud.

On a dry weight basis adsorbed potassium concentrations ranged from 84 to 154 μg/g in the control lakes, and from 77 to 217 μg/g in the fertilized lakes (Table 4). Following fertilization, some fertilized lakes exhibited a greater amount of potassium than was

TABLE 4. *Adsorbed potassium in bottom muds*[1]

Date	Control lakes				Fertilized lakes							
	North Twin		South Twin		West Lost		Hemlock		Lost		Section Four	
	Wt	Vol	Wt	Vol	Wt	Vol	Wt	Vol	Wt	Vol	Wt	Vol
June 1949...	84	2.3	—	—	217	9.3	140	5.0	112	8.5	217	—
Aug. 1949...	97	2.5	126	3.5	168	7.2	126	4.5	84	6.4	154	—
Aug. 1950...	140	3.8	140	3.9	203	8.7	112	4.0	126	9.6	196	143
Aug. 1951...	154	4.2	98	2.7	199	8.6	168	6.1	77	5.9	112	82
Average..	119	3.2	121	3.4	197	8.5	137	4.9	102	7.6	169	112

[1] Wt quantities are μg K/g of dried mud; vol quantities are μg K/cc of wet mud.

present before fertilization, but others exhibited smaller amounts. It cannot be concluded, therefore, that adding potassium artificially to these natural waters necessarily increases the potassium concentrations in the adsorbed phase of bottom muds.

On a wet (by volume) basis the average quantities of adsorbed potassium were higher in the fertilized lakes than in the control lakes, the former ranging from 4.9 to 112 μg/cc and the latter from 3.2 to 3.4 μg/cc (Table 4). Since differences in adsorbed potassium in bottom muds could not be attributed to the fertilization program (as indicated above), the difference between fertilized and unfertilized lakes in adsorbed potassium must be due to some other factor. Potassium amounts, when measured in terms of units per unit volume of wet mud, vary in such a manner that the greater the content of solids (dried muds) in the wet muds the greater the concentration of potassium (Table 5). It may be concluded, therefore, that the adsorptive rate of potassium by bottom muds is a function of the amount of solids per unit volume of lake bottom muds. The amount of solids per unit volume of wet muds however varies inversely with the percentage of organic matter within the muds. Therefore, since potassium concentration by volume is a function of percentage solids, and since percentage solids varies inversely with percentage organic matter, it must be true that with an increase in concentrations of organic matter in muds there is a decrease in concentration of potassium per unit volume in the muds. This conclusion is considered at greater length in the following discussion.

TABLE 5. *Concentrations of dried muds and adsorbed potassium in wet muds and percentage organic matter in dried muds*

	North Twin	South Twin	West Lost	Hemlock	Lost	Section Four[1]
Dried muds g/cc	0.027	0.028	0.043	0.036	0.076	0.730
Av. μg K/cc	3.2	3.4	8.5	4.9	7.6	112
Av. org. matter %	58	59	50	53	43	6

[1] Values listed are for the clay-marl-sand sediments of 1950 and 1951. In 1949 the organic matter content of the pulpy-peat sediment was 41%.

Some time during the spring of 1950, a considerable "chunk" of the bank, and some of the bottom of Section Four Lake collapsed and literally dropped out of sight. What was once land now lay submerged beneath the waters of the lake. The extent of the area of subsidence was about 200 feet along the lake shore and about 25 feet back from the original shore line. This event was probably brought about by the dissolution of underlying limestone bed rock with the result that there was not sufficient support for the overlying glacial drift. The collapse of the bank of Section Four Lake lends support to the concept that this and other lakes of this region are of limestone sink origin.

As a result of the "catastrophic" subsidence of this land mass in 1950, the pulpy peat muds in the bottom of Section Four Lake were completely covered by a new deposition of a clay-marl-sand mixture. Samples of the pulpy peat bottom muds collected in 1949 contained 41% organic matter; in the summer of 1950, following the "catastrophy", there was 6% organic matter in the muds (Table 5). Data are not available on the quantity of dry mud per unit volume of wet mud for the muds of Section Four Lake prior to the summer of 1950; however, muds collected in the summer of 1950 consisted of 0.730 g of dry solids per cc of wet mud (Table 5). Values of pulpy peat muds from the other lakes investigated ranged from 0.027 to 0.076 g/cc, and it seems likely that the pulpy peat muds of Section Four Lake contained similar percentages of solids prior to their burial by the inorganic sediments. The new muds of Section Four Lake thus contained less organic matter and a lower water content per unit volume than the muds formerly present.

Muds collected from Section Four Lake in August 1950 contained 196 μg of adsorbed potassium per g of dry mud (Table 4). It can be seen therefore that this mud consisting predominately of inorganic materials (only 6% organic matter) had potassium concentrations of the same order of magnitude as were present in the pulpy peat muds

of the other lakes and in the pulpy peat muds of Section Four Lake prior to its "catastrophe." Thus, on a dry-weight basis organic and inorganic muds contained essentially the same amounts of adsorbed potassium. When compared on a volume basis however, wet organic muds did not contain the same amounts of potassium as wet inorganic muds. In the organic muds the adsorbed potassium ranged from 2.3 to 9.6 μg/cc of wet mud, whereas in the highly inorganic muds of Section Four Lake the potassium contents ranged from 82 to 143 μg/cc of wet mud (Table 4). Thus, quantities of adsorbed potassium per cc of wet inorganic muds were many times greater than concentrations in wet organic muds. Expressed in another way, for each square-centimeter of surface area of lake-bottom muds, the predominately inorganic muds contained more adsorbed potassium than the predominately organic muds. This would be true for mud strata of the same thickness. Therefore, it may be hypothesized that when soluble potassium is put into lakes, the potassium will go out of solution more rapidly in oligotrophic type lakes which have clay-marl bottom muds than in eutrophic-type lakes which have rich organic bottom muds.

It is of special interest to note the quantities of potassium put into the lakes as compared with amounts found in the muds of the lake bottoms. Amounts put into the lakes, in terms of grams of potassium per square meter of lake surface area, were: West Lost 0.68, Hemlock 1.75, Lost 3.19, and Section Four 4.28. Amounts of adsorbed potassium found in bottom muds may be expressed in terms of amounts per square meter of a layer of mud one cm thick ($g/m^2/cm$). These values (Aug. 1951) were: West Lost 0.086, Hemlock 0.061, Lost 0.059, and Section Four 0.82. Thus, for each gram of adsorbed potassium computed to be present in a layer of mud one cm thick and one meter square in area, there were put into the lakes the following amounts: West Lost 7.9 g; Hemlock 28.7 g; Lost 54.0 g, and Section Four 5.2 g. According to these figures, from 5.2 to 54.0 times as much potassium was put into the lakes as was found to be present in the muds.

Since the potassium fertilizer put into the lakes could not be found in epilimnial waters in August 1951, and since only a small fraction of it was detected in hypolimnial waters at this time, and inasmuch as there was no evidence of it accumulating in the bottom muds, there remains unanswered the question, "Where did the added potassium go?" Two possibilities have not yet been explored. Potassium fertilizer may accumulate in high concentrations at the mud-water interface. Improved sampling devices and techniques should be used to determine if this actually happens. In the present study the muds at this interface could not be collected without mixing them with some of the sediments under the mudwater interface. Also, there is the possibility that most of the added potassium was converted into insoluble compounds, either organic or inorganic. Further studies are needed to answer these questions.

SUMMARY

1. Commercial fertilizer containing potassium was applied to 4 of 6 trout lakes. Applications were made during the summers of two successive years. Two of the lakes were used as controls.

2. Samples of epilimnial and hypolimnial waters, and of bottom muds, were collected from the 4 fertilized lakes and from the 2 control lakes. These samples were analyzed for their potassium contents. Samples were collected during the two years of fertilization and for one year following fertilization.

3. During the first year, epilimnial potassium contents increased in the fertilized lakes following applications; the increases were roughly in proportion to amounts of potassium added. Potassium contents in the control lakes did not increase during this time.

4. During the second year, potassium contents increased in both control and fertilized lakes. Increases in control lakes are thought to have been of allochthonous origin, because of abundant rainfall that year. Excessively high increases in both

phosphorus contents and phytoplankton growths in the control lakes of that year lend support to this hypothesis.

5. Abundant growths of phytoplankton occurred in the presence of only medium potassium concentrations (about 1.0 ppm). This leads to the conclusion that potassium is not a critical factor in limiting productivity in most lakes.

6. Increases in hypolimnial potassium contents in the fertilized lakes seem to have been due to the fertilization program.

7. No increases in contents of adsorbed potassium in the bottom muds could be attributed to fertilization, although the use of a different mud sampling technique might have shown that adsorbed potassium contents of the mud-water interface did in fact increase as a result of fertilization.

8. Muds high in organic matter content and high in water content had less adsorbed potassium per unit volume than muds high in inorganic materials and low in water content. This relation leads to the hypothesis that soluble potassium is more readily removed from lake waters in oligotrophic lakes which have marl-clay substrates than in eutropic-type lakes which have organic-rich substrates.

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NOTES AND COMMENT

An Improved Method for the Determination of Preserved Plankton Volumes[1]

With the increasing use of plankton volumes as a parameter in marine biology it seemed desirable to eliminate certain difficulties in the usual method of volume determination by filtration (see Yentsch and Hebard 1957). The objections to this method are: (1) getting rid of interstitial water during filtration and, (2) loss and mangling of delicate specimens making later identification difficult if not impossible. It is thought that the method presented here will eliminate these objections with no increase in time or labor.

The principle is based on the colorimetric method of volume determination of blood plasma utilizing a coloring material which is not taken up by the blood cells or, in this case, the preserved plankton organisms. The procedure consisted of making up the plankton sample with sea water and formalin to a known volume in a volumetric flask, preferably as small as convenience would allow for later sampling. The sample was shaken and allowed to settle for five minutes (more for very small organisms), leaving a supernatant fluid only slightly cloudy with animal fragments and various debris. A small sample was withdrawn from the supernatant, measured as a blank in a Klett-Summerson colorimeter, and returned to the flask together with 1 ml of a stock color solution (diluted india ink). The flask was then shaken again and allowed to settle for five minutes after which a second measurement was made with the Klett. A simple computation gave the volume of contained plankton:

$$\text{plankton volume} = (V_F + 1) - \frac{(V_S + 1)K_S}{(K_T - K_B)}$$

where:

V_F = volume of flask containing plankton
V_S = any convenient but known volume of water in which 1 ml of stock coloring has been diluted for measurement, in this case 100 ml
K_S = Klett units, measurement of V_S
K_B = Klett units, for blank supernatant from plankton sample made up to V_F
K_T = Klett units for supernatant from plankton sample plus 1 ml stock color.

Some comparisons of the filtration and colorimetric technique are given in Table 1. It will be noted that volumes determined colorimetrically are less than those by filtration, which is attributed to the interstitial fluid remaining after filtration. The differences between filtration and colorimetric determinations are generally comparable to those obtained by Yentsch and Hebard (1957) who compared filtration volumes with those of plankton air-dried for 24 hours. Filtration volumes in this report were obtained by measuring the total sample volume, vacuum filtering through fine bolting silk, and then subtracting the volume of the filtrate from that of the original sample.

TABLE 1. *Comparison of plankton volumes (in ml) by two methods*

Filtration	Colorimetric
85	80
12	10
77	69
82	79

Greater accuracy in the colorimetric method is obtained by keeping the volume to which the plankton sample is made up

[1] Contribution No. 230 from the Bermuda Biological Station. Contribution No. 903 from the Woods Hole Oceanographic Institution.

small and by using a more concentrated color stock. A convenient concentration was found to be approximately 2 ml of Higgins American India Ink diluted to about 200 ml with water. The addition of 1 ml of this stock produced no objectionable color in the plankton sample and, indeed, could hardly be distinguished from some samples whose contents had been leached by standing several months in preservative. All samples used were preserved with formalin neutralized with borax.

REFERENCES

YENTSCH, C. S., AND J. F. HEBARD. 1957. A gauge for determining plankton volume by the mercury immersion method. Journ. du Cons., **22:** 184–190.

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BOOK REVIEW

FASSETT, NORMAN C. 1957. **A manual of aquatic plants.** With a Revision Appendix by Eugene C. Ogden. University of Wisconsin Press, Madison. ix + 405 pp. $6.50

The first 362 pages of this book are identical with the edition published by McGraw-Hill in 1940. Pages 363 through 384 constitute a Revision Appendix prepared by E. C. Ogden partly in collaboration with Fassett before his death. The index has been completely reworked to include the Revision Appendix as well as the original text.

Fassett's book was originally intended, and remains, primarily for the north-eastern quarter of the United States. Species from outside this area are not included, although of course many species of aquatic plants are wide ranging, which makes this book usable in all parts of the country. Fassett's definition of aquatic plants is much broader than that in the book by Muenscher[1], in that it includes the woody plants, macroscopic algae, and many species of herbaceous plants that ordinarily might not be considered aquatic.

All items of revision are contained in the appendix prepared by Ogden. This has obviated the expense of resetting type for the entire book, but it has resulted in the inconvenience of having to refer constantly to the appendix. It would be best for the user to recopy all the items of revision into the text of the book itself, even though this would be quite a chore.

The Revision Appendix is concerned chiefly with the many small details involved in bringing the nomenclature into agreement with present-day usage. Significant references since 1940 on the various taxa are included, along with the addition of a few new species reported from this geographic region. And, finally, substitute keys for identification are provided for a few genera, including *Potamogeton*, *Echinodorus*, and *Callitriche*.

In spite of the inconvenience of using the Revision Appendix, this book will continue to be our most useful reference on aquatics, particularly for the northeastern states.

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[1] Muenscher, Walter Conrad. 1944. Aquatic plants of the United States. Comstock Publ. Co., Ithaca, N. Y. x + 374 pp.